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NOTICE

The Indian Journal of Agricultural Science

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ORIGINAL ARTICLES

VARIETAL CHARACTERS AND CLASSIFICATION OF THE RICES OF EASTERN BENGAL.

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(With Plates I-VII, and four text-figures)

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Preface.

The following account of the varietal characters and classification of Bengal rices is based on observations which have been made at Dacca during the course of the last twenty-two years. Experience has shown that a complete varietal classification of Bengal rices is almost an impossible task, owing to the tremendous range of variation which exists. It could be done only by the co-operative efforts of many workers. But a classification of some sort, however imperfect, is a necessity, and the following account of varietal characters is put on record both as a contribution to the subject, which might otherwise be lost, and for the information of other workers.

Introduction.

The area dealt with in the following account of the varietal characters of rice comprises the Dacca, Chittagong and Rajshahi Divisions of Bengal, without the Hill-Tracts, as shown in the map (Plate I). Throughout this area rice is the staple food crop, covering an area of 127,908,000 acres in 1930-31, and is grown almost entirely for consumption.

Work on the classification and selection of rice varieties suitable for these tracts has been carried on at Dacca as the central station of East Bengal for about 22 years, as well as at several of the smaller District farms, such as Barisal in the Barisal Sunderbans, Comilla in Chittagong Division, Mymensingh in Dacca Division, and at Rangpur, Bogra, Rajshahi, Dinajpur and Pabna in Rajshahi Division.

Agricultural crops.

Within the area as defined above there are five main, distinct crops grown, sown and harvested under different conditions and at different periods of the year.

These are :—

(1) Highland *Aus*, sown broadcast on highland in April-May and harvested in August-September.

(2) Transplant *Aman*, sown in seed-beds in June, transplanted in semi-lowland in July-August, and harvested in November-January.

(3) Lowland *Aus*, sown broadcast in lowland in February-March, harvested in August-September. These varieties grow in 5 ft. to 6 ft. of water, and are harvested in water.

(4) Lowland *Aman*, sown as No. 3 but on still lower areas and harvested in December-January, when the flood waters have dried up. Some of these varieties can grow in 30 ft. of water.

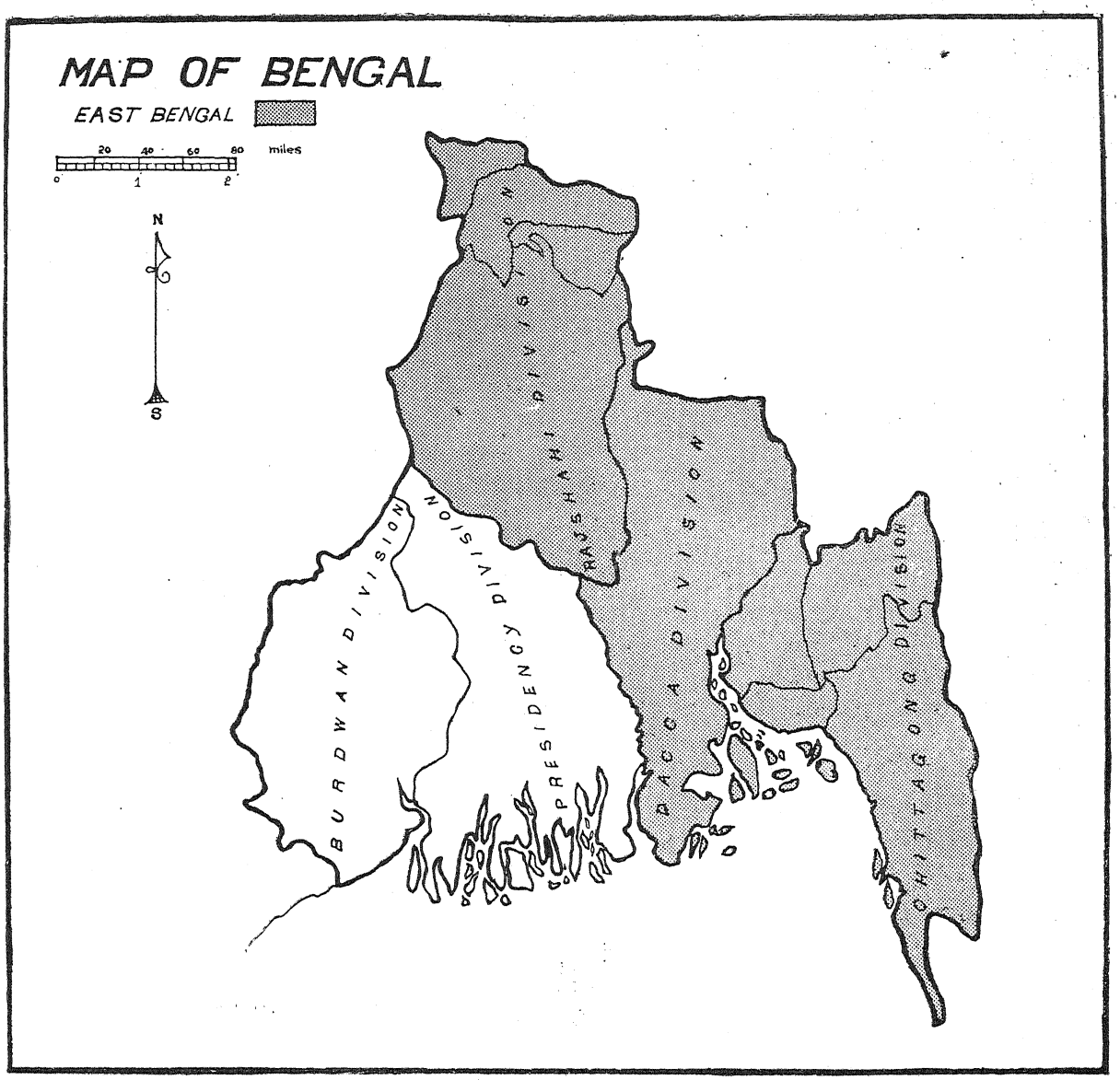
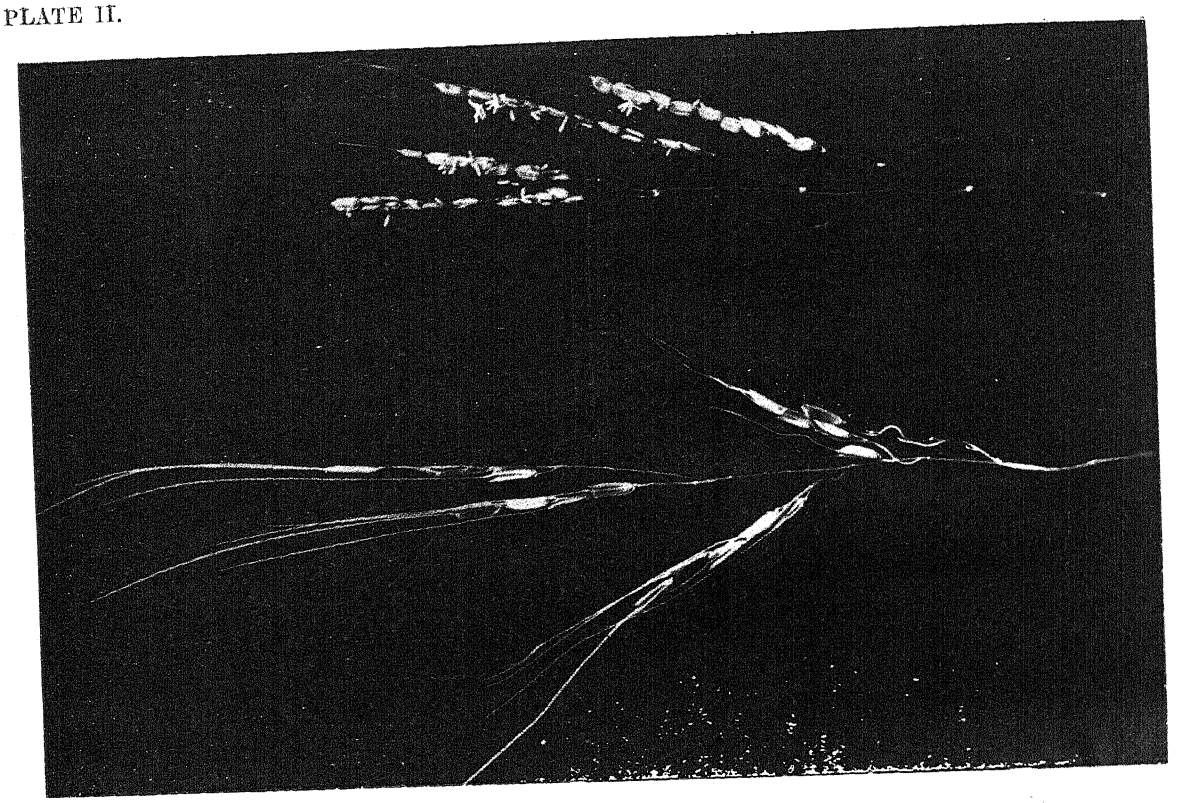
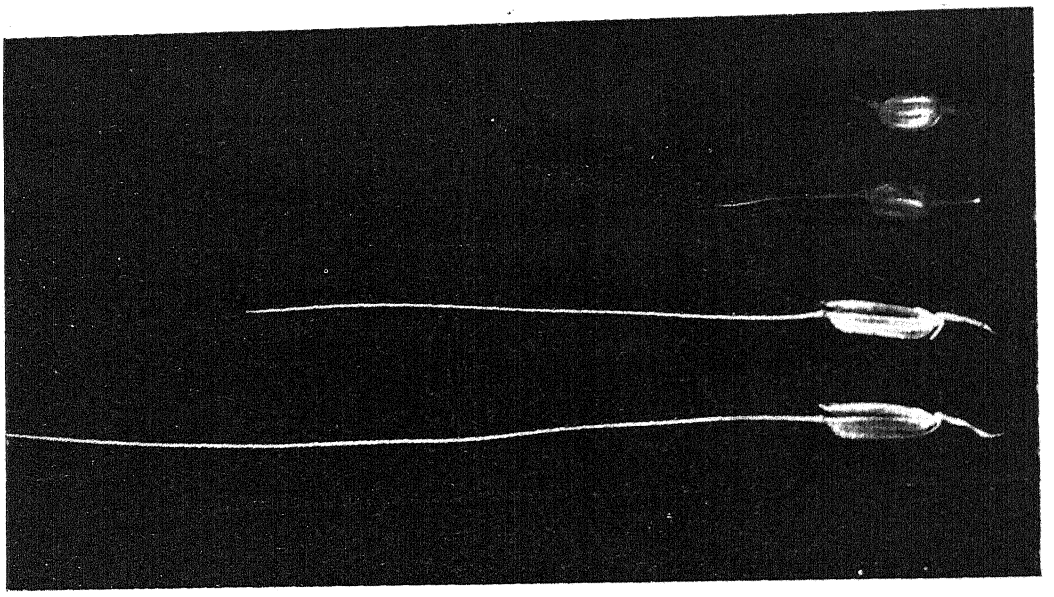

MAP OF BENGAL
EAST BENGAL
20 40 60 80 miles
0 1 2
N
S
RAJSHAHI DIVISION
PRESIDENCY DIVISION
BURDWAN DIVISION
DACCA DIVISION
CHITTAGONG DIVISION

PLATE II.





VARIETIES OF WILD RICE.

(5) *Boro*, sown in October in seed-beds, transplanted in December on the lowest areas of all which do not dry up even in the cold weather, and harvested about March.

Each of these crops has its own distinctive varieties, or rather physiological types, which will grow only under their own conditions. These types may be morphologically alike, but physiologically they are totally distinct. The following account of the varietal characters deals with the varieties and types of Nos. 1 and 2 only, which are well defined groups, covering the largest areas. They, together with the so-called *boro* rices, may be regarded as the short-stemmed paddies, with upright stems, as opposed to the deep water paddies, classes (3) and (4), which cannot stand upright but whose stems float in the water and grow rapidly in length as the flood increases. All these varieties undoubtedly derive from the same wild parent, which grows commonly in the *bhils* of East Bengal to-day. This is evidenced by the fact that they all cross readily with the wild rice and with each other, and have the same number of chromosomes.

Collection of material.

The material comprised in the following account has consisted of samples of seed of the chief varieties as known by the cultivators collected at various times by the members of the Botanical Staff, by the District Agricultural Officers, and by the help of the Collectors of the various districts and their subordinate officers. These samples of seed have been grown at Dacca and type selections of single plants made therefrom. Altogether 856 types of transplant *Aman*, and 931 types of highland *Aus*, classed into 540 varieties, are described in the following classification. The scheme of classification here described has been in use at Dacca for many years, but has not been hitherto published.

Description of varietal characters.

Colour in the vegetative parts of rice.

A large number of varieties belonging to each of the different agricultural groups are characterised by the presence of soluble coloured pigment in various parts of the growing plant. This pigment occurs in several shades, ranging from faint traces of pink through red, violet, and blue to deep purple. In winter rice,

the colour is generally more intensified than in highland early (*Aus*) rices, and it reaches the highest degree of concentration in the submerged stems and leaf-sheaths of deep-water winter rices.

The pigment is generally found in a diffused state in the epidermal cells or in the tissues surrounding the bundles, assuming an appearance of coloured streaks, and may also occur scattered in the general parenchyma.

The presence of colour in different organs of the plants has been found to be due to a single factor, or to several interacting factors. Frequently the colours are grouped into patterns or systems, due to the fact that the pigment is distributed in different combinations in the leaf-sheath, internode, node, outer glumes, inner glumes, apiculus and stigma, and the whole pattern may behave as a single unit in segregation as if due to a single unit factor, or to the same interacting factors. Another view is that the constituent parts of these patterns are due to different factors closely linked [Hector, 1922]. The evidence in favour of this view lies in the fact that rare instances of the breakage of these patterns have been found. On crossing coloured varieties of various patterns with green varieties, the colour has always been found dominant, and in the F_2 generation various Mendelian ratios such as 3:1, 9:7, 27:37, have been found of 'colour' : 'absence of colour'.

The presence of various combinations of colours forms a very distinguishing feature in rice, and can be utilized in drawing up a system of classification based on colour distribution. It must be noted, however, that in rice no correct judgment of the relationships which constitute varieties can be based on outward resemblance only. For a precise knowledge it is essential to study the behaviour of the pigment in heredity. Again, the range of colour variation is so great that any attempt to make a comprehensive classification based on outward similarities only will be far from satisfactory. All that can ordinarily be done is to take up each variety separately and describe it in detail.

The pigment in rice is located chiefly in the following organs or parts of organs :—

(1) Leaf.

- (*a*) Leaf-sheath.
- (*b*) Pulvinus.
- (*c*) Auricle.
- (*d*) Ligule.
- (*e*) Lamina.
- (*f*) Flag.

(2) Internode.
(3) Outer glumes.
(4) Inner glumes.
(5) Apiculus.
(6) Stigma.
(7) Awns.

The colours observed in the mature inner glumes and grains are of a different nature from the pigment found in the different parts of the growing plant, and they have generally been found to segregate independently of the pigment in the vegetative parts.

The nature and relationship of the pigment in the different parts of the rice plant mentioned above and its behaviour on segregation are briefly described below :—

1 (a) Leaf-sheath.—The seedlings of almost all coloured rices develop the colour in the form of a streak running through the first leaf-sheath (cotyledon), immediately after germination. This serves as a useful diagnostic character at the earliest stage of growth, and has been found useful in genetic studies, as it offers an easy means of separating coloured individuals from colourless in F_2 and subsequent generations at a very early stage.

The colour of the first leaf-sheath varies in intensity in different varieties.

During subsequent stages of growth, the colour of the leaf-sheath develops, and the development varies greatly. The pigment may be located at the base of the leaf-sheath only, or over a greater part of the surface, outside, inside or both. It is generally found in the epidermal cells, or in the tissues surrounding the bundles, assuming an appearance of coloured streaks, but may also occur scattered in the general parenchyma. It has also been observed that the colour develops early or at a later stage, and may persist till the maturity of the plant or disappear sooner. The most intense colours, however, are always found to persist throughout the life of the plant, fading away only towards maturity. The colour in the leaf-sheath has always been found dominant to its absence, and to segregate in F_2 in a simple 3 : 1 ratio, 9 : 7 ratio, 27 : 37 ratio and in a 15 : 1 ratio. Thus the colour in the leaf-sheath is due to a simple factor or to two or more interacting factors.

Graham [1913] states that all rices which have a coloured leaf-sheath have a dark-coloured apiculus to the glume. Our observations, on the other hand, show that there is no such definite correlation between the colour in the leaf-sheath and the colour in the apiculus. The evidence in support of this view is that a large number of varieties belonging to the highland *Aus* group are characterised by the presence, at one stage or other of plant growth, of pigment in a very light shade, without the faintest trace of colour in any other part of the plant. There is no

doubt that this is a genuine colour although it is of a fleeting nature. This type by mistake is apt to be classed with the absolutely colourless, because the presence of colour in the leaf-sheath in such cases is very apt to escape detection, unless the plants are very critically examined during different stages of growth.

Thadani [1930] also states that the association between the colour in the leaf-sheath and apiculus does not exist in Sind rices, and out of seventeen varieties of coloured rices examined by him he has found four varieties having a coloured leaf-sheath with no colour in the apiculus.

Sethi and Durga Datta [1928] have found in the United Provinces that all the varieties of rice which possess a deep purple-coloured leaf-sheath invariably possess the coloured apiculus, but in a few kinds where leaf-sheath colour is light or dull purple the apiculus is colourless. This agrees with our observations in Bengal rices. In our observations, however, spread over a very large number of highland *Aus* and transplant *Aman* varieties, the number of varieties belonging to the latter type is quite considerable.

Thadani states that in Sind rice all varieties with a green leaf-sheath have a white grain (rice). In our observations, on the other hand, we have found that there is no such correlation in Bengal rices.

1 (b) Pulvinus.—The pulvinus, the cushion-like structure at the junction of leaf-sheath and leaf blade, may be coloured or colourless. When coloured it varies in the degree of intensity in different varieties. The colour in the pulvinus has always been found associated with the colour of the leaf-sheath, but the converse is not true.

1 (c) Auricle.—The auricles, the two ear-like appendages projecting from the margins of the pulvinus, are also coloured or colourless. Various shades of colour are found in different varieties. This character has been found to stick to the pulvinus and behave in a similar way on segregation. The presence of colour in the auricle is associated with the presence of colour in the leaf-sheath, but the converse is not true.

1 (d) Ligule.—Like the pulvinus and auricle this is a character of minor importance. The ligule may be coloured or colourless, and in the former case the leaf-sheath has always been coloured. The intensity of colour in the ligule in different varieties varies from faint traces to deep purple.

1 (e) Lamina or leaf blade.—The lamina is usually green, but when coloured the colour is generally located in the margins and in the midrib. Occasionally, however, in varieties in which colour is present in an intense form in other parts, a sprinkling of purplish colour is observed throughout both surfaces of the lamina. Varieties of this type can easily be detected from a distance.

1 (f.) Flag.—The flag is the terminal member of the leaf-system and may be considered as analogous to the bract of dicotyledons. It is usually shorter and broader than the leaves below, and varies considerably in size in different varieties. The sheath of this leaf encloses the inflorescence before emergence. After emergence of the inflorescence the flag assumes a position almost parallel to it, and the tip of the flag may reach the same level or a higher or a lower level, depending mainly on the exsertion of the inflorescence. The length and breadth of the flag may vary considerably even within the same variety. The position of the flag with respect to the main axis, on the other hand, is a fairly constant character. There is a certain amount of variation in this position during the progress of growth, and therefore it has been found necessary to note this character at a particular stage of the plant growth when it is fairly constant, *i.e.*, when the inflorescence has fully emerged and is exserted to the farthest limit. In certain varieties which have been noted for strong straw, the flag makes an acute angle with the stem, and the position is maintained throughout the life of the plant. It therefore appears that this erect position of the flag is associated with the strength of the straw. In other cases, however, the flag assumes different positions, such as horizontal (at right angles to the main axis), drooping (obtuse angle), etc., and is a distinguishing feature of different strains. As regards colouration, there in no difference between it and the normal leaves.

(2) Internode.—The internode, the part of the culm between two nodes, may be coloured or colourless. In the former case the colour occurs diffused in the epidermis or in the general parenchyma, or is confined to the bundle sheaths in the form of coloured streaks, or in various combinations of these. Like the leaf-sheath the colour in the internode may appear at any stage of plant growth, early or late, and be temporary or permanent. The colour in the internode has always been found dominant to its absence, and may be due to one, or to the interaction of two or more factors. In actual crosses studied so far, the colour has been found to segregate in a 3 :1 ratio, 9 :7 ratio and 27 :37 ratio. In one instance of cross between two types having no colour in the internodes (Chittagong 25 × Dacca 13), colour in the internode was found to appear in the F_2 and subsequent generations. This shows that colour in the internode in certain cases may remain inhibited and be liberated on crossing.

(3) Outer glumes.—The outer glumes may be coloured or colourless. When coloured, the colour bears no constant relation to the colour of the inner glumes and stigma. The presence of colour in the outer glumes has always been found associated with presence of colour in the leaf-sheath, but the converse is not always true. The colour may develop early, or at a later stage of flowering, and generally grows fainter during maturity. It is fairly constant and forms a useful differentiating

character. This colour in the outer glumes has been found to behave in Mendelian fashion, giving simple 3 : 1, as well as 9 : 7 ratios on segregation, in crosses so far examined.

(4) *Inner glumes.*—The inner glumes in the majority of cases are free from coloured pigment. In many cases, however, a sprinkling of colour is found especially in highly-coloured varieties with a deeply coloured apiculus. In such cases the colour has been found to spread from the base of the apiculus downwards over the glume surface. When the pigment is present, it may be associated with the colour in the outer glumes, and in such cases the inner glumes behave in a similar way with the outer glumes on segregation.

The mature inner glumes, or the husk of the rice grain, present different shades of colour, ranging from yellow through red, buff, orange-brown to deep purple in different varieties. Occasionally a piebald pattern is found in which the upper portion of the glumes is red of some shade, and the base pale yellow, or the red colour may be located in the middle portion of the glumes only with the upper and lower portions yellow. Another pattern is also noticed in a large number of varieties in which the colours are confined to the furrows, the ridges or the nerves being free, or possessing a colour of a lighter shade than that of the furrows. This type of piebald colouration of the husk is generally more prevalent in coarse-grained rices. The colour of ripe inner glumes is of a different nature from that of the soluble pigment, and has not so far been found to bear any relation to the colour in the vegetative parts. In genetic studies of the colour in the ripe glumes, definite Mendelian ratios have been obtained in some cases. Black colour has been found to be dominant over yellow, and yellow over red, segregating in 3 : 1 ratio. In one case of a cross between yellow and red glumes, the segregation was into 9 'yellow' : 6 'yellow and red' : 1 'pure red.'

(5) *Apiculus.*—This is the apex of the spikelet formed by the union of the tips of the inner glumes. In the majority of varieties the apiculus does not show any pigmentation, and has generally a colour indistinguishable from that of the inner glumes. But in varieties where pigment is present in the apiculus, the colour consists of a spot varying in intensity from vermilion to deep purple (almost black), and is generally noticeable just after the complete emergence of the inflorescence from the sheath. The colour in the apiculus may, however, develop at a later stage of flowering. This colour in some cases is of a transient nature, fading away towards maturity, or disappearing altogether in very lightly coloured varieties. In intensely coloured varieties, however, the colour of the apiculus persists till the maturity of the spikelet, and forms a distinguishing character. It has been found that all varieties having coloured apiculus have also colour in the leaf-sheath and outer glumes, but the converse of such a correlation is not always true. In

PLATE III.

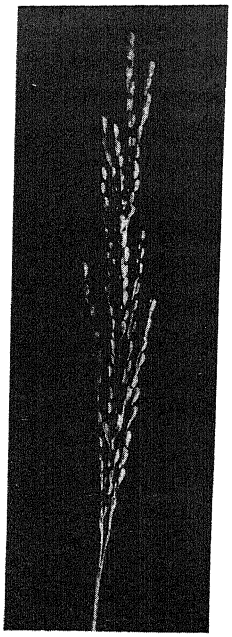


Fig. 1.

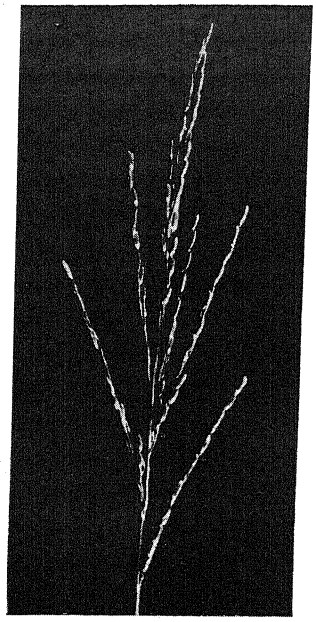


Fig. 2.

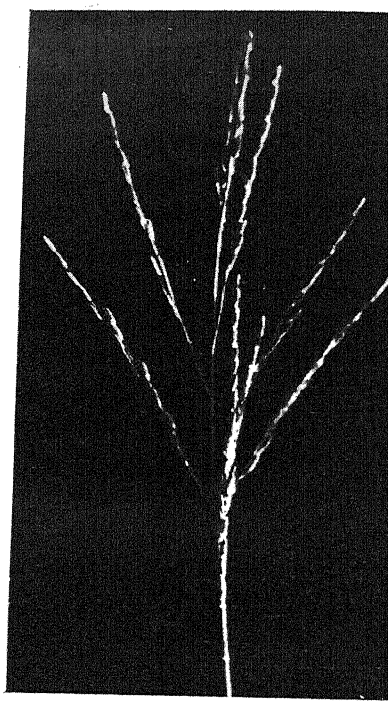


Fig. 3.

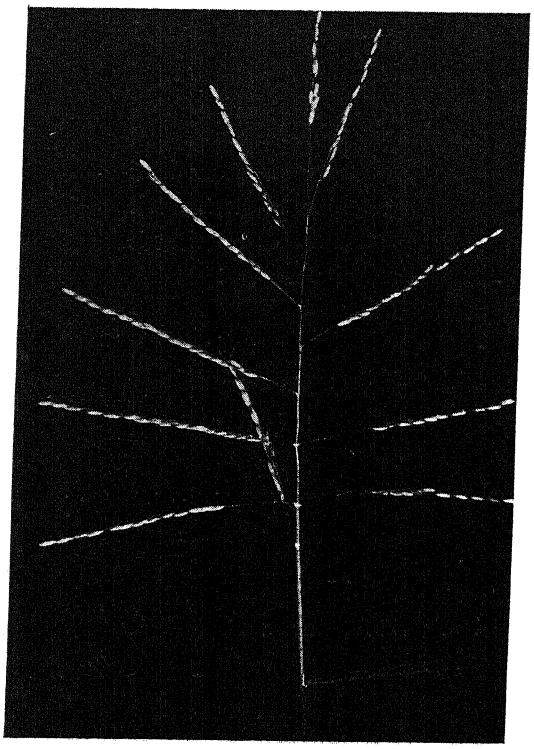


Fig. 4.

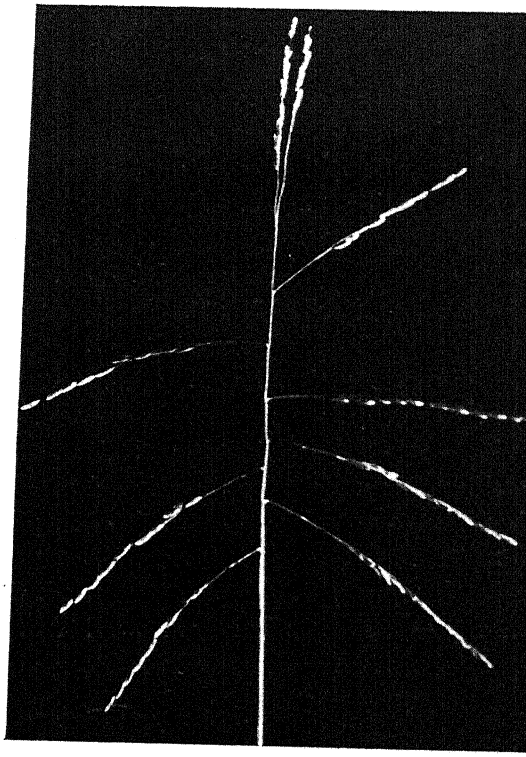


Fig. 5.

INFLORESCENCES OF RICE SHOWING ANGLES OF PRIMARY BRANCHES.

genetic studies the colour in the apiculus has been found to be due to a single factor or to two or more interacting factors, giving 3 : 1, 9 : 7, 27 : 37 and 15 : 1 ratios.

(6) *Stigma.*—The stigma of the rice flower may be white or dark purple. Occasionally, however, a colour of a lighter shade is found in some varieties. The colour of the stigma is a very constant character, and has been found useful for diagnostic purposes. In earlier work, Bengal rices, with reference to the distribution of colour in leaf-sheath, apiculus and stigma, were broadly classified as follows [Hector, 1916]:—

1. Leaf-sheath, apiculus of the glumes, and stigma coloured.
2. Leaf-sheath and apiculus of the glumes coloured but stigma colourless (white).
3. Apiculus and stigma coloured but leaf-sheath colourless.
4. Apiculus of glumes only coloured.

Later observations, however, have proved that the last two classes do not actually exist, but a third class exists of "leaf-sheath only coloured". The colour in the leaf-sheath in the last two classes is of so faint and fleeting a nature that it escaped detection in earlier observations. By closer observation, a definite colour relationship of stigma with that of leaf-sheath has been established. When the stigma is coloured, the leaf-sheath also is invariably coloured, but the converse is not true. In crosses, the colour in the stigma has been found to be due to a simple factor or to several interacting factors, segregating in different ratios in F_2, such as 3 : 1, 9 : 7, 27 : 37, 81 : 175 and 15 : 1 in different varieties so far experimented with.

(7) *Awn.*—Quite a large number of varieties of rice in Bengal are characterised by the presence of awns. Awns have generally a colour uniform with that of the tip of the glume, and may be white (colourless), pink or reddish purple.

Inflorescence.

The inflorescence of rice is a panicle borne on the topmost internode, the peduncle.

The panicle consists of a main axis, the rachis, which is a much-branched structure, bearing primary and secondary branches. According as the branches form a small or large angle with the main rachis, the inflorescence may be close and compact, or open and loose [Plate III]. The disposition of the inflorescence depends on the strength of the rachis during the reproductive and maturity stages. It may be erect, half-curved and drooping, or sharply curved. Like the rachis, the primary branches may also be erect, curved or drooping. In some cases the lower branches of the inflorescence may be erect, while the upper ones have a drooping disposition. The number of primary branches in inflorescence is a very variable character,

Gammie [1908], in distinguishing the varieties of rice of the Bombay Presidency, utilized the average number of primary branches as the basis of distinction, but in Bengal this character is a very variable one and cannot be used as a basis of classification. The secondary branches are much smaller in size, and on these branches the spikelets are attached by their pedicels, which may be long or short. The spikelets are usually situated singly on their pedicels, but in a few varieties are clustered in groups of two or three. The length of the panicle in different strains varies to a large extent and is considerably influenced by extraneous factors such as fertility, spacing of the plant, etc. It is measured from the first scar, *i.e.*, the ciliate ridge on the peduncle, up to the tip of the topmost spikelet. Within each pure strain, also, there does not seem to exist any constancy between the length of the central or main panicle, and those of the lately devoloped side culms. The length of the earhead in Bengal varieties has been found to vary from 16·6 to 28·0 cm. in highland rices, and from 14·0 to 42·0 cm. in transplanted *Aman* rices. Generally speaking the length of the inflorescence is associated with the number of branches. A long panicle has proportionately fewer branches than the short panicle. The general erect and close disposition of the short panicle together with its proportionately more number of branches, make this type appear more compact.

Peduncle.

This is the uppermost internode of the plant bearing the panicle. A part of this internode is enclosed by the sheath of the flag leaf and that part which emerges out of the sheath is the peduncle proper. The extent to which this emergence takes place is measured by the distance from the junction of the flag blade and sheath to first scar. The first scar is situated at the junction of the peduncle and panicle, and in the majority of cases it bears the lowest branches of the panicle. According to the extent of emergence of the first scar from the junction of the flag blade and sheath the peduncle may be described as :—

(1) Inserted	*i.e.*, when the scar does not emerge out of the sheath (Plate IV, fig. 1).
(1) Inserted-exserted (or "just exserted"=0).	*i.e.*, when the scar is just visible above the flag sheath (Plate IV, fig. 2).
(3) Far exserted . . .	*i.e.*, when the scar is far above the leaf-sheath (Plate IV, fig. 4).
(4) Exserted	*i.e.*, when the scar occupies an intermediate position between the "just exserted" and the "Far exserted" ones (Plate IV, fig. 3).

Another possible extreme condition is the complete absence of emergence of the whole inflorescence from the leaf-sheath. This we have not met with so far in

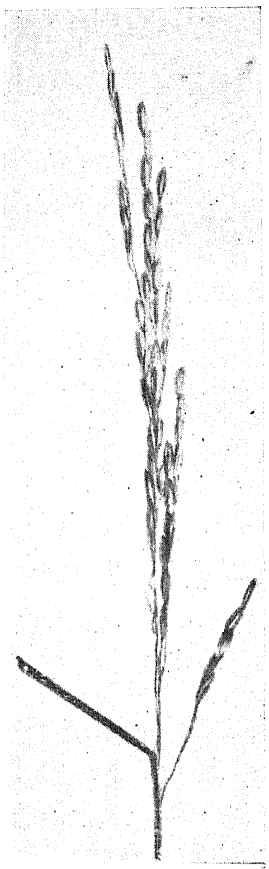


Fig. 1.

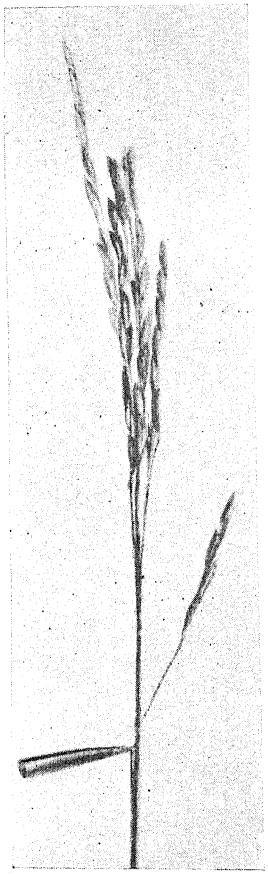


Fig. 2.



Fig. 3.

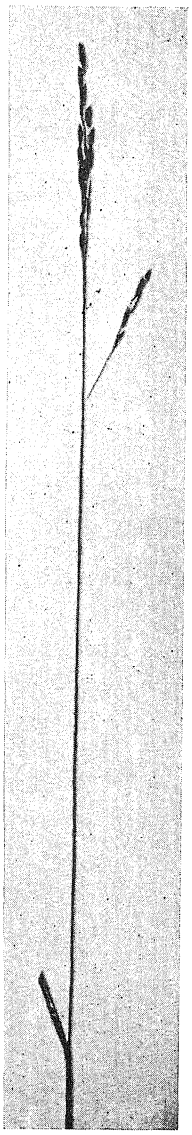


Fig. 4.

TYPES OF EXSERTION OF PEDUNCLE.

PLATE V.

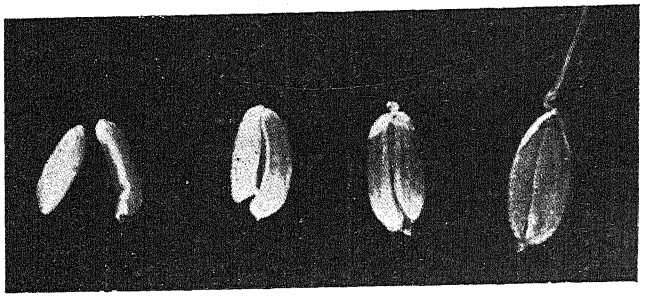
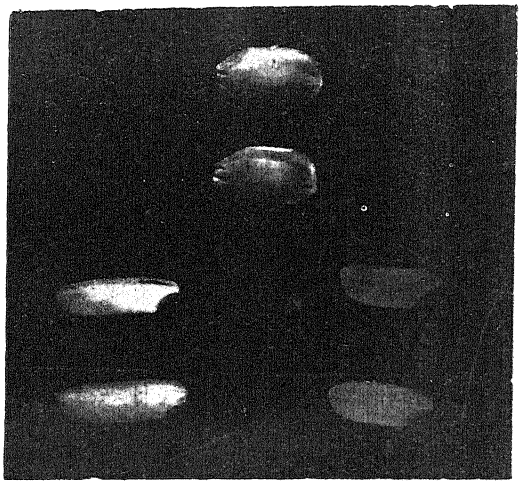


Fig. 1.—Double grained paddy.

Abdominal white



Glutinous. Translucent.

Fig. 2.

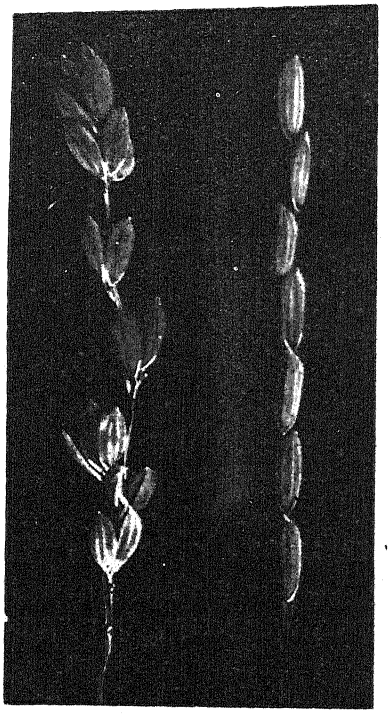


Fig. 3.—Arrangement of spikelets.

Bengal rices. A partial emergence of the inflorescence is however met with in some of the early strains of highland *Aus* and transplanted *Aman* varieties. Sethi and Saxena [1930] say that this complete absence of emergence of the inflorescence is very useful as a protection against the ravages of the rice hopper called 'gundi' (*Leptocorisa varicornis* F.) which sucks away the milky juice of the young spikelets.

The extent of emergence of the peduncle from the flag sheath varies in different varieties. The average exsertion of an individual strain is fairly constant, although there exists a certain amount of variation, within a narrow limit, between the central and the side shoots of any pure strain. The fine varieties are characterized by a longer exsertion of the peduncle than the coarse ones. The emergence of the peduncle from the flag sheath is affected by environment. It has been observed that in rich soil the exsertion of the peduncle is less as compared to the same grown in poor soil; also in years of poor growth there is greater emergence than in years of good growth.

The extent of exsertion in the Bengal rices under study varies on the average from 0-13·2 centimetres in highland *Aus* varieties and 0-42·0 centimetres in transplanted winter varieties.

Arrangement of spikelets.

Ordinarily the spikelets of rice are borne singly on the pedicels of the secondary branches of the panicle. Besides this, another condition is found in some varieties, in which the spikelets are arranged in groups of 2 to 7 at definite intervals on the rachis, assuming a peculiar interrupted appearance (Plate V, fig. 3). The number of spikelets in each cluster is not constant, even on the same plant. This condition is known as "clustered." Individuals belonging to this group are very few. In the scheme of classification, varieties may be grouped into (1) solitary and (2) clustered, based on the fact that these two conditions are distinct, definite and hereditary characters.

Outer glumes.

With respect to the length of the outer glumes Bengal varieties can be classified into (1) short-glumed (normal), (2) long-glumed (winged). The majority of the varieties of rice are short-glumed, and there are only a few varieties which belong to the long-glumed class. On account of the winged appearance of the latter class they commonly go under the name of "Pankhiraj" in Bengal.

The outer glumes (Plate VI, fig. 1) in rice are always sterile and empty, and their function is not definitely known. But it seems that they play a part in keeping the inner glumes in position during pollination, when the opening and closing of the flowers take place. It is probable that on account of their glossy nature the outer glumes protect the essential organs and the embryo in the early stages of growth by warding off outside moisture.

It has been observed in crossing that the short outer glumes (normal) are dominant over the long outer glumes (winged), and segregation is generally in a 3 : 1 ratio.

Mature inner glumes.

The inner or flowering glumes of rice are two in number, and are usually about three times the size of the empty outer glumes. Of these, the third glume is usually five-nerved and encloses, near the margins, the fourth glume, known as the pale, which is usually smaller in size than the third glume and is three-nerved. These nerves constitute the ridges, and the portions between them are known as furrows. It seems that there is some association between the extent of ridging and the size and shape of the grain. It has been observed that most of the coarse-grained varieties are characterized by the presence of prominent ridges. The mature inner glumes constitute the hull of the grain. They vary in thickness in different varieties. From an economic point of view a thin hull is a distinct advantage, as it facilitates milling, and gives a less proportion of husk. The two inner glumes enclose the kernel, either loosely or tightly.

The colour of the mature inner glumes is constant, and presents a useful diagnostic character which can be utilized for classificatory purposes. The colour may be uniform or variegated, and shows a wide range, extending from the common pale yellow to a deep black. For practical purposes the Bengal rices have been classified into the following five groups, based on the colour of the mature glumes :—

(1) Yellow.
(2) Red.
(3) Black.
(4) Piebald red.
(5) Mottled.

Class (1) includes all shades of yellow, ranging from the common pale yellow to golden yellow.

Class (2) includes many shades of red, ranging from light red to deep red, through buff, orange, brown, etc.

Class (3) includes light black to deep black, through various shades of grey and purple.

Class (4) includes those rices having a pattern in which the upper portion of the glumes is red of some shade and the base is yellow; or the red colouration may be located in the middle portion of the glume only, with upper and lower portions yellow.

Class (5),—in this class the colour of the glumes is chiefly confined to the furrows, the ridges or the nerves being almost free, or possessing a colour of lighter shade than that of the furrows.

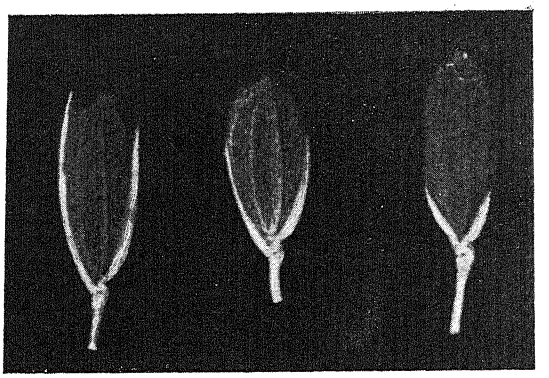


Fig. 1.—Outer glumes.

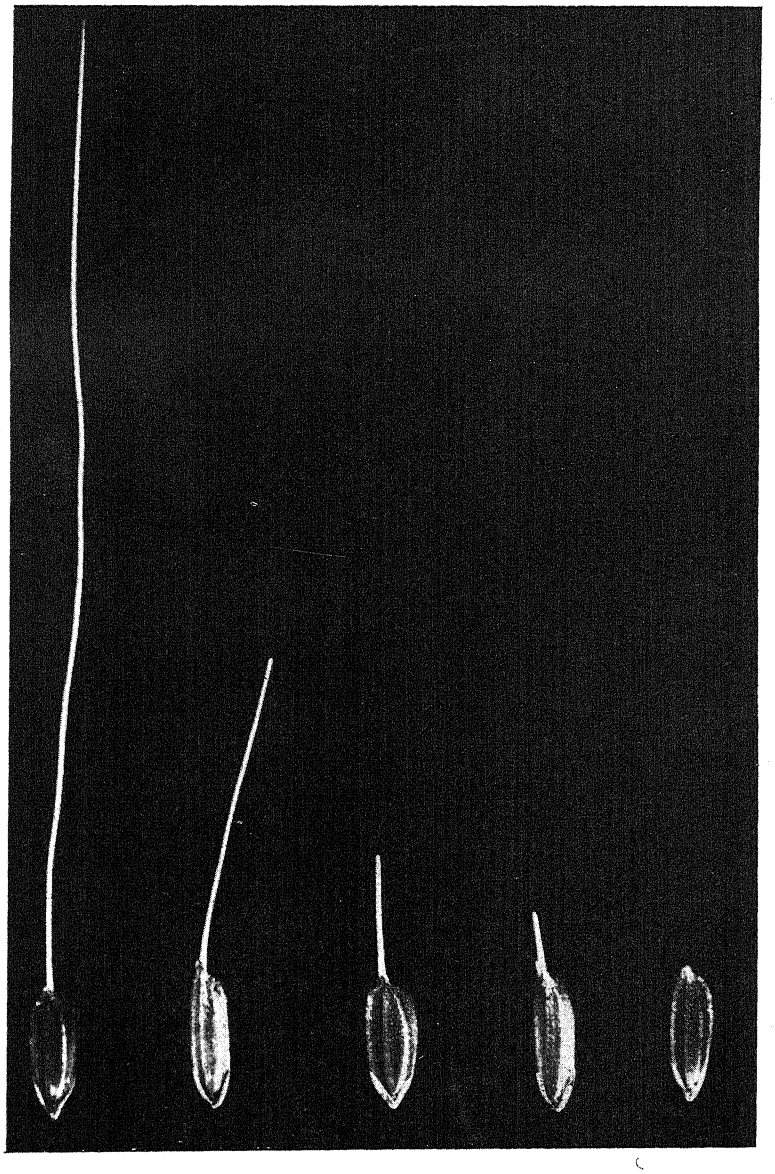
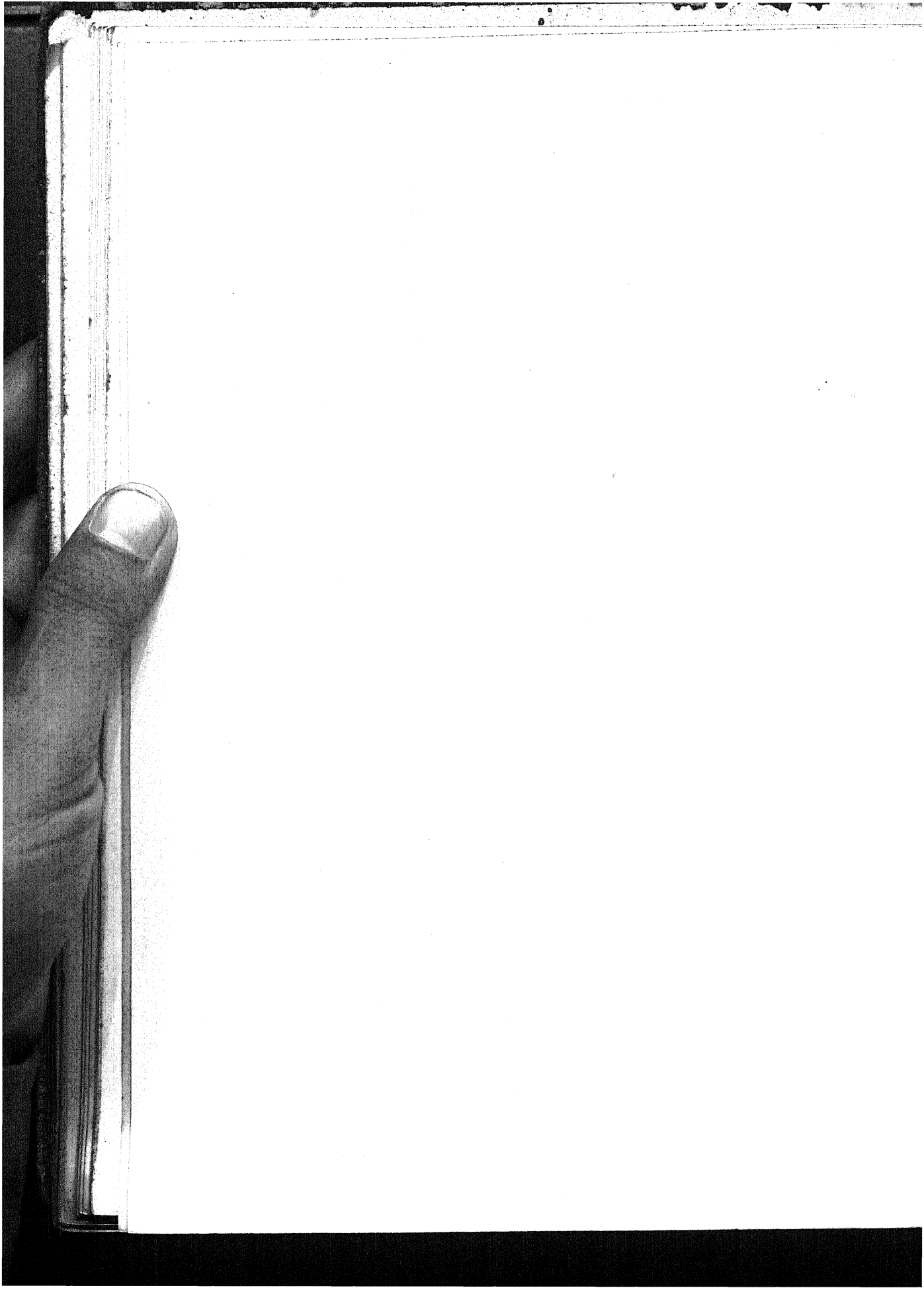


Fig. 2.—Awns.



Awns (Plate VI, fig 2).

The presence or absence of awns in rice forms an important basis for classificatory purposes. It is a character which is handed down from the ancestral wild paddies to the cultivated varieties, and is an important hereditary character. Next to wild paddies, the deep-water paddies are generally characterized by the presence of long awns. The proportion of awned to awnless varieties diminishes as the paddies proceed towards upland conditions. Thus in the highland broadcast paddies the proportion of awned to awnless is the least. This points to the fact that water plays a part in the development of awns. In fact some of the apparently awnless highland varieties have been found to develop awns when grown in lower situations.

With respect to presence or absence of awns rice can be classified into (1) awned and (2) awnless. The awned group can further be subdivided into (1,a) long-awned and (1,b) short-awned, according to the length of awning. It may be pointed out that the feature of awning is a variable character and appears to be influenced by environmental factors. Even within the same variety the variation in awning is not inappreciable. In some varieties the awn may be almost unmeasurable, and often escapes detection. In some of the short-awned varieties it has been noticed that the awns are not always present on every spikelet of the panicle. Generally the spikelets of the topmost region bear awns, leaving the lower spikelets free. On the other hand it has been found in certain cases that there is no regular system, but the awns may occur at random on any spikelet of the panicle. Copeland [1924] observes "awns seem at least sometimes to be associated with general vigour, perhaps in that they were a character of the ancestral wild rice, and the breeder who would get rid of them might conceivably do this through the elimination of vigour. It is hardly questionable that as a general average, awned rices are heavier producers than awnless varieties". This does not agree with our observations in Bengal. Vigour may sometimes be associated with awns, but there is no definite correlation between awns and yield. In fact most of the high-yielding Bengal rices belong to the awnless group. A certain amount of correlation seems to exist between coarseness of the spikelet and the presence of awns. Generally speaking awns are found mostly among coarse varieties, but the presence of awns among fine varieties of rice is not unknown and the common wild rice of Bengal, which has long awns, has a very fine grain.

Although awned varieties are preferred in some localities to safeguard the crop against the ravages of birds and wild animals, the presence of awns is a drawback for milling purposes, and for handling at the time of harvest and subsequent operations.

In Bengal rices under study, the length of awns varies in different varieties from minute tips to as much as $3\frac{1}{2}$ or 4 inches.

The inheritance of awns has been found to be a complicated matter and has not been fully investigated. In one cross long awns were found definitely dominant, in another recessive.

Husked grain—colour.

The husked grain, known as the kernal, is the final product of the rice plant. It varies in colour, consistency, size and shape in different varieties. The colour of the kernel is a very constant character and is hardly affected at all by outside factors. It is located in the thin pericarp, and can be easily removed during milling, leaving a white rough rice. In some cases the kernels are prominently ridged and offer considerable resistance at the time of milling, so that a portion of the coloured material is left adhering to the finished product. The presence of colour in the kernel is considered a disadvantage, as it depreciates the value of the rice. In Bengal, as in other countries, preference is given to white rice, but the cultivators in general have a special liking for coarse, red rices, in the belief that they are sweeter, more nutritious and have a distinctive flavour. The complete removal of the pericarp deprives the grain of its vitamin content, and an exclusive diet of such rice is supposed to give rise to many neurotic diseases. The colour of the kernel is a hereditary character, and on crossing with colourless (white) varieties, it has been found to behave in a Mendelian fashion, segregating generally in 3:1 ratio, though there are complications.

The colour varies in different varieties, ranging from white to charred black, through different shades of red (Plate VII, fig. 1). According to the colour of the kernel, the Bengal rices may be broadly classified into the following three groups :—

(1) White	Includes dull to glossy white.
(2) Amber	Including various lighter shades of red.
(3) Red	Including various deeper shades of red *e. g.* vermillion terra-cota, etc.

Besides these, three more conditions of an exceptional nature have been met with. These are :—

(*a*) Sprinkled (mottled)---Here the kernel is not uniformly coloured but has a sprinkling of red pigment and appears mottled or dotted.

(*b*) Piebald.—Here also the kernel is not uniformly coloured. There is a deeper shade of colour along the margins, with a lighter shade in the centre, or the central portion may be almost free from colour.

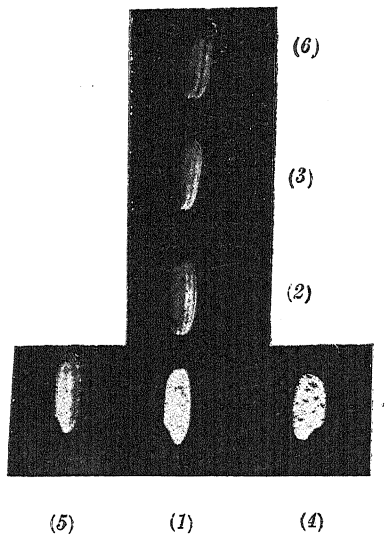


Fig. 1.—(1) White. (2) Amber. (3) Red. (4) Mottled. (5) Piebald. (6) Charred black.

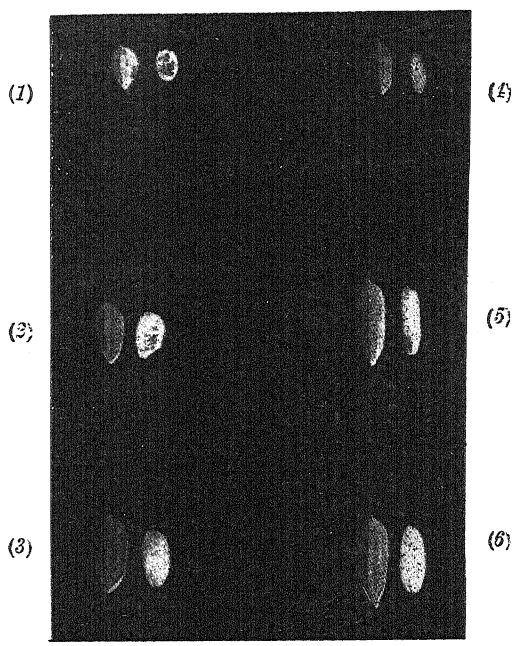


Fig. 2.—(1) Oval fine. (2) Oval medium. (3) Oval coarse. (4) Oblong fine.
(5) Oblong medium. (6) Oblong coarse.

(*c*) Charred black.—Here the colour of the kernal is of a very deep shade, formed by the combination of deep red and purple pigments. The colour is not uniformly spread over the entire surface of the kernel, the embryonic region being of a lighter colour.

Since the above three conditions are of rare occurrence, the varieties falling under them are for convenience grouped in the transplanted *Aman* rices under the amber or red classes, according to the intensity of the colour. In the great majority of the highland *Aus* rices the colour of the kernel is generally of a dull nature, and therefore the red rices amongst the *Aus* varieties have not been classed separately, according to the shade of colour, into red and amber.

Husked grain—consistency.

The kernel of rice consists mainly of an endosperm with the embryo situated laterally near the base. During the process of formation the endosperm passes through three stages before it attains maturity, depending mainly upon the proper supply of moisture. The first stage is characterised by a white liquid substance composed of strachy material, the "milk stage". As the growth of the endosperm proceeds, there is an accretion of more material into the substance of the endosperm. It becomes more viscous and at last reaches the final stage, when the endosperm hardens and assumes its normal nature. The final stage is the ripe stage of the endosperm. According to the composition and texture of the endosperm at the ripe stage, Bengal rice may be broadly classified into :—

(1) Non-glutinous or translucent.

(2) Glutinous.

1. Non-glutinous endosperm is composed of starch in its most concentrated form. It is hard, brittle and translucent. It gives a uniform blue reaction with iodine. From an economic point of view, non-glutinous rices are more important than the glutinous ones, as they mill better and do not break to any considerable extent during hulling and polishing. They also present a more attractive appearance. On account of their hard texture they stand transportation better. Some of the non-glutinous varieties present a fractured appearance of the endosperm on husking. This in many cases is due to an over-ripe or over-dry condition, but in certain cases this condition has been found to be varietal. Such varieties are not suitable for milling.

The endosperm of all non-glutinous, translucent rices becomes opaque during germination, probably due to the hydrolysis of the starch.

2. Glutinous endosperm is quite distinct from the non-glutinous, as it is comparatively soft and opaque, and is composed of dextrin. When the grain is cut open the endosperm presents a chalky white appearance, supposed to be due to dextrin, a partially hydrolysed product of starch. The term glutinous is really a misnomer, as glutinous rice has nothing to do with gluten proper. Perhaps the term has been adhered to on account of the sticky mass a glutinous rice is converted into on boiling, which is the chief characteristic of gluten. It gives a red colouration with iodine.

The glutinous rices are not commonly cultivated in Bengal, but may occasionally occur as mixtures with the non-glutinous rices. Economically they do not form so distinct and valuable a group as in some other countries, such as China and Japan, where they are much prized for confectionery.

Besides these two distinct conditions of the endosperm, quite a large number of varieties of rice are characterized by the presence of a chalky white spot on the dorsal side at the centre (belly) of the endosperm. This has been described by Kikkawa [1912] as "abdominal white" and is found associated with many of the coarse-grained rices of Bengal. This white-bellied portion on examination shows the presence of starch in a comparatively loose form, mixed with a small proportion of dextrin. It may be due to lack of uniformity in filling, during the process of development. This 'abdominal-white' character is sometimes confused with an almost similar condition met with in many grains, due to immaturity. The immature grains, however, when cut open present a chalky appearance in the core of the grain, and not in the abdomen. Graham [1913] states that the 'abdominal-white' condition is an indication of immaturity of grain, and therefore cannot be recommended as a basis for classification. Our observations on the other hand prove beyond doubt that a genuine abdominal white is a hereditary character, and is not much influenced by environmental factors. It is therefore an important diagnostic character. The abdominal white is found in both the non-glutinous and glutinous varieties of rice. In glutinous rice the presence of the abdominal white is masked by the more or less similar nature of the rest of the endosperm, and can be detected only with difficulty. In non-glutinous rices, on the other hand, the abdominal white when present is very prominent, and further sub-division into the following two sub-classes is therefore possible, *viz.*:—

(1) Translucent	having a corneous endosperm throughout.
(2) Abdominal white	having a corneous endosperm with a prominent crescent-shaped chalky abdomen.

In heredity the translucent character has been found to be dominant over glutinous and abdominal white, and behaves in a simple Mendelian fashion on segregation. The glutinous or the abdominal white characters, being endosperm characters, are always one generation ahead of the plant which bears them, and on crossing with the translucent they are found in F_1 and subsequent generations to be present in the same plant in the proportion of 3 translucent : 1 abdominal white or glutinous, as the case may be.

The kernels of all rices, if husked immediately after harvest, stick together on boiling, irrespective of their consistencies, *i.e.*, no matter whether they are non-glutinous or glutinous. In the non-glutinous rices, however, this property gradually diminishes by storage. The kernels of a non-glutinous rice after about three months' storage do not stick together on boiling with the proper amount of water, but remain separate. In the glutinous rices, on the other hand, the kernels do not lose the property of sticking together with any amount of storage.

In Bengal, table rice is prepared by two distinct processes, and is known accordingly as :—

(1) Parboiled (Siddha).
(2) Sun-dried (Atap).

In the first process the unhusked grain is dried, then steamed under pressure till the husk bursts, re-dried and then husked in the ordinary way. The rice prepared by this method is largely used by the people. In the sun-dried process, on the other hand, the unhusked grain, after a few sunnings, is husked direct. The sun-dried and parboiled rice of the same variety differ considerably in flavour, taste and digestibility on cooking. The parboiled rice is easily digestible, as most of the albuminous nutrient material is removed during the process of par-boiling, and has less flavour and taste than the sun-dried (Atap). Parboiling facilitates hulling, as the kernel is loosened from the husk during the process. It also makes the grain less liable to breakage.

Grain—size and shape.

In Bengal, rice is grown mainly for local consumption, only a small fraction, the so-called Bengal and table rices, being exported. Since the agrarian population is in the majority, the major portion of the production is consumed by the cultivators themselves, while a small proportion finds its way to the local markets for meeting the needs of the rich, middle and labouring classes. In these markets rice

is valued according to its quality. The quality again is mainly determined by the size, shape and fragrance of the grain. Rices characterized by the presence of fragrance command the highest price, but since they are very few in number with a very limited market, they have been left out of any special consideration in the scheme of classification given below. The classification suggested herein deals mainly with the size and shape of the grain, which are very constant characters being little influenced by cultural and environmental factors, and are important determiners of quality.

The size and shape of the grain are determined by its length, breadth and thickness. These characters in a hulled grain correspond closely to the same in its unhulled state. But considerable differences are observed in different varieties in the actual measurement of length, breadth and thickness in the unhulled and hulled condition, depending on whether the kernel loosely or tightly occupies the space within the husk. It has also been observed that in those varieties where the space within the husk is tightly occupied by the kernel, the husk as a rule is thin. Such types of grains are of great economic importance, on account of the proportionately higher return of rough rice obtained from them.

The grain can be measured in three dimensions, *viz.*:—length, breadth and thickness, easily by a screw micrometer. In the Bengal rices under study the length of the unhulled grain varies from 5·15 mm. to 11·27 mm., breadth from 1·97 mm. to 3·73 mm. and thickness from 1·61 mm. to 2·59 mm. whereas in the hulled state the length varies from 3·72 mm. to 8·23 mm., breadth from 1·62 mm. to 3·29 mm. and thickness from 1·45 mm. to 2·36 mm.

To start with, rices can be broadly classified into oval and oblong according to the proportion of length to breadth of an unhulled grain. All rices having a ratio up to 3 have been grouped as oval, following Kikkawa [1912], and those above 3 as oblong. This is a rough and ready method of determining the quality of rice, particularly in the unhulled state, when it is known as paddy. But for a more precise assessment, it is necessary to base the classification of rice, on the measurement of grain in its hulled state, known as rough rice, which is the product that mainly finds its way to the market. The thickness of the hulled grain along with its breadth, play an important part in determining quality, and accordingly rices are further classed into fine, medium and coarse, based on the ratio of the length to the product of breadth and thickness of the hulled grain, with respect to particular lengths. The scheme followed in the classification of Bengal rices with respect to size and shape of the grain is appended.

GROUP A.—OVAL-GRAINED.

When the length is 3 times the breadth or under.

(1) Fine—With grain length	*a* 3·5 to 3·99 and	$\frac{L}{B \times T}$	0·6 and over.
,,	*b* 4·0 to 4·49 ,,	,,	1·0 ,, ,,
,,	*c* 4·5 to 4·99 ,,	,,	1·2 ,, ,,
(2) Medium— ,,	*a* 4·0 to 4·49 ,,	,,	0·8 to 0·9
,,	*b* 4·5 to 4·99 ,,	,,	1·0 to 1·1
,,	*c* 5·0 to 5·49 ,,	,,	1·4 to 1·5
,,	*d* 5·5 to 5·99 ,,	,,	1·5 to 1·6
(3) Coarse— ,,	*a* 4·0 to 4·49 ,,	,,	0·7 and less.
,,	*b* 4·5 to 4·99 ,,	,,	0·9 ,, ,,
,,	*c* 5·0 to 5·49 ,,	,,	1·3 ,, ,,
,,	*d* 5·5 to 5·99 ,,	,,	1·4 ,, ,,
,,	*e* 6·0 to 6·49 ,,	,,	1·4 ,, ,,
,,	*f* 6·5 to 6·99 ,,	,,	1·6 ,, ,,
,,	*g* 7·0 to 7·49 ,,	,,	1·6 ,, ,,
,,	*h* 7·5 to 7·99 ,,	,,	1·6 ,, ,,

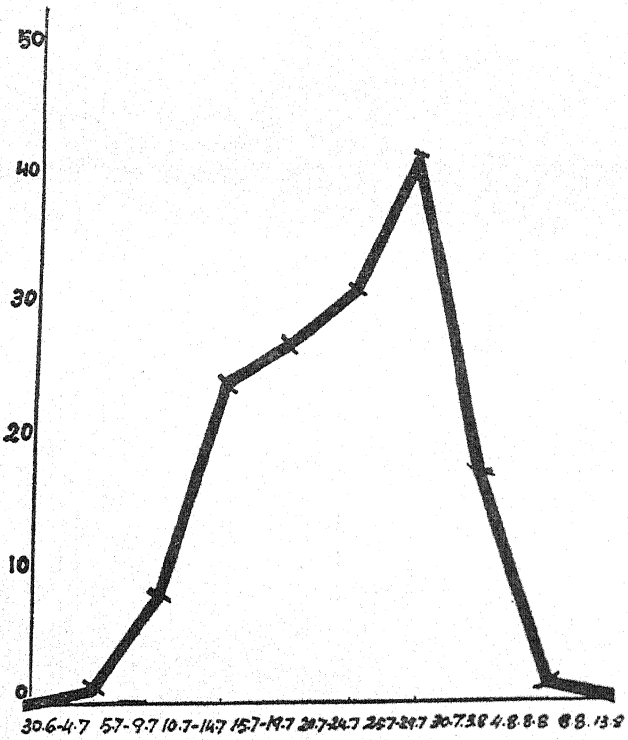
GROUP B.—OBLONG-GRAINED.

When the length is more than 3 times the breadth.

(1) Fine—With grain length	*a* 4·5 to 4·99 and	$\frac{L}{B \times T}$	1·6 and over.
,,	*b* 5·0 to 5·49 ,,	,,	1·7 ,, ,,
,,	*c* 5·5 to 5·99 ,,	,,	1·7 ,, ,,
,,	*d* 6·0 to 6·49 ,,	,,	1·9 ,, ,,
,,	*e* 6·5 to 6·99 ,,	,,	1·9 ,, ,,
,,	*f* 7·0 to 7·49 ,,	,,	2·0 ,, ,,
,,	*g* 7·5 to 7·99 ,,	,,	2·0 ,, ,,
(2) Medium— ,,	*a* 5·0 to 5·49 ,,	,,	1·4 to 1·6
,,	*b* 5·5 to 6·0 ,,	,,	1·5 to 1·6
,,	*c* 6·0 to 6·49 ,,	,,	1·6 to 1·8
,,	*d* 6·5 to 7·0 ,,	,,	1·6 to 1·8
,,	*e* 7·0 to 7·49 ,,	,,	1·7 to 1·9
,,	*f* 7·5 to 7·99 ,,	,,	1·7 to 1·9
,,	*g* 8·0 to 8·45 ,,	,,	2·0 to 2·1
(3) Coarse— ,,	*a* 5·0 to 5·49 ,,	,,	1·3 and less.
,,	*b* 5·5 to 5·99 ,,	,,	1·4 ,, ,,
,,	*c* 6·0 to 6·49 ,,	,,	1·5 ,, ,,
,,	*d* 6·5 to 6·99 ,,	,,	1·5 ,, ,,
,,	*e* 7·0 to 7·49 ,,	,,	1·6 ,, ,,
,,	*f* 7·5 to 7·99 ,,	,,	1·6 ,, ,,

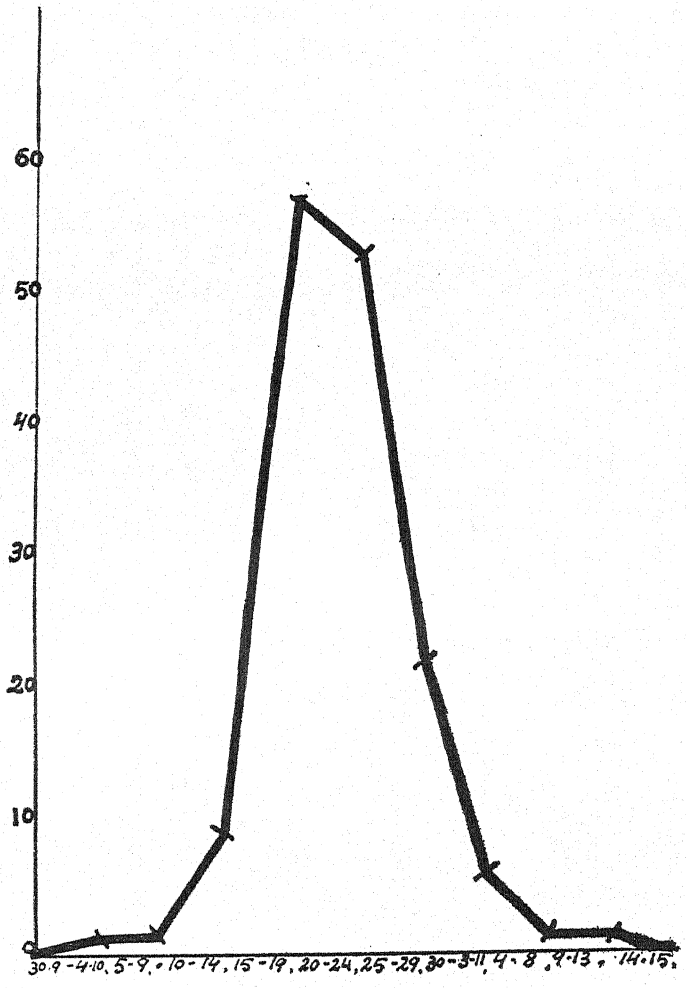
Field characters.

Date of flowering :—In Bengal rices under study at Dacca, the highland broadcast *Aus* group is usually sown in April and May depending upon the conditions of moisture in the soil. The flowering usually commences from the last week of June and continues till the first week of August in the different strains, with the modal point at about the 30th July, the range being from 30th June to 4th August (Fig. 1). In the transplanted winter group, on the other hand, the seeds are sown in nurseries in May-June and the seedlings are transplanted in puddled fields about 5 or 6 weeks later. The flowering in this group usually commences from the end of September in the early strains and continues till the middle of November in the very late ones with the mode at about 19th October, the range being from 30th September to 15th November (Fig. 2).



Date of flowering.

Fig. 1.—Curve showing the range of flowering of 931 types of highland *Aus* paddies. Average of 3 years, 1927-29.



Date of flowering.

Fig. 2.—Curve showing the range of flowering in 857 types of transplanted *Aman* paddies. Average of 3 years, 1927-29.

If we take these two groups together we find that the flowering ranges from the last week of June to the middle of November, with a distinct gap of about 6 weeks, extending from the second week of August to the last week of September, during which normally there is no paddy in existence which flowers under the conditions of East Bengal. This fact is further borne out by actual hybridization experiments. In a

cross between a variety of highland *Aus* paddy (Kataktara, Dacca No. 2) and a late transplanted winter paddy (Indrasail, Dacca No. 1) it has been observed, curiously enough, that in the F_2 generation the progeny segregated into two distinct groups, one flowering during the normal *Aus* period and the other during the normal *Aman* period, presenting almost a similar gap as obtains normally under the Dacca conditions between the flowering of *Aus* and *Aman* paddies. As an exception, however, a very few plants, numbering about seven, flowered during this gap, but they were characterized invariably by the presence of a large percentage of sterile grains.

It is the general belief that the earliness or lateness of a variety is determined by the date of flowering. This is, however, not always the case. It is the interval between the flowering and maturity, along with the actual date of flowering, which determines whether a variety is early or late, and in other words it may be said in general that the total duration from sowing to maturity is the criterion for the earliness or lateness of a particular strain.

The actual date of flowering in any strain of *Aus* paddy varies directly with the date of sowing. Within certain limits a strain sown earlier or later will correspondingly flower earlier or later, the most salient feature being that the total duration from sowing to ripening for any particular strain is fairly constant. This character of flowering in *Aus* has been aptly termed by Mitra [1932] as " periodically fixed ".

In the transplanted winter paddies, on the other hand, the actual date of flowering is constant, so much so, that a particular strain sown and transplanted at different dates in different years flowers almost on the same date with very little variation. No matter whether sown early or late, a variety belonging to this group will flower almost at a fixed time, and it has been observed that even a difference of a month or so in transplanting delays the flowering only by about a week. This mode of flowering has been described by Mitra as ' timely fixed '. In the case of transplanted paddies, therefore, from the actual date of flowering one may form an approximate idea as to whether a strain will be early or late, but for a precise judgment it must be read along with the duration from flowering to ripening.

Though much influenced by cultural and seasonal variations, the date of flowering is a definite hereditary character.

Duration.—By duration is understood the total period of time required by a crop from sowing till its maturity. This life period in the case of different varieties of *Aus* paddies examined ranges from 90 to 124 days (Fig. 3), whereas in the case of transplanted *Aman* paddies, it is from 138 to 183 days from the earliest to the latest (Fig. 4). Again, the total duration of a particular variety is fairly constant,

fluctuating within limits, the fluctuation being influenced by extraneous factors, such as time of sowing, fertility of the soil, moisture conditions, temperature and season. Such a wide range in the duration is very important from an agricultural point of view. Varieties having different life periods are suitable for varying conditions. In higher situations, where water accumulates to a very limited extent, and in poor soils, varieties having short duration will generally do well, whereas in intermediate or low situations, with a fair or ample supply of water, and in rich soils, varieties having comparatively longer durations will be most suitable.

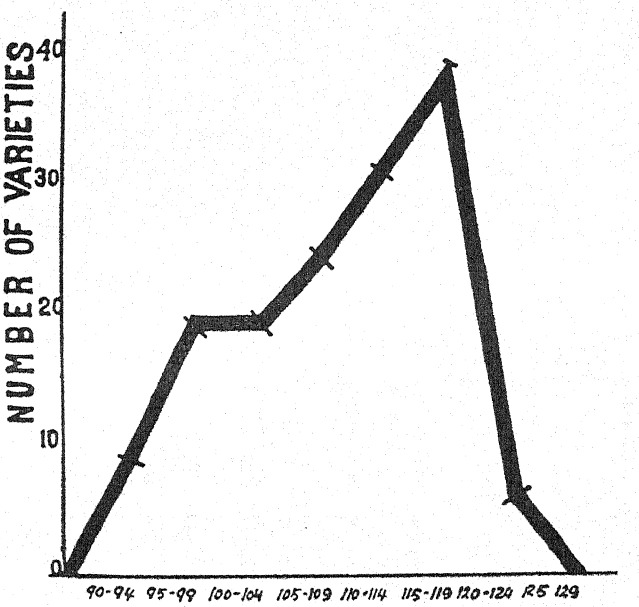


Fig. 3.—Curve showing the total duration (from sowing to ripening) of 931 type of highland *Aus* paddies. Average of 3 years, 1927-29.

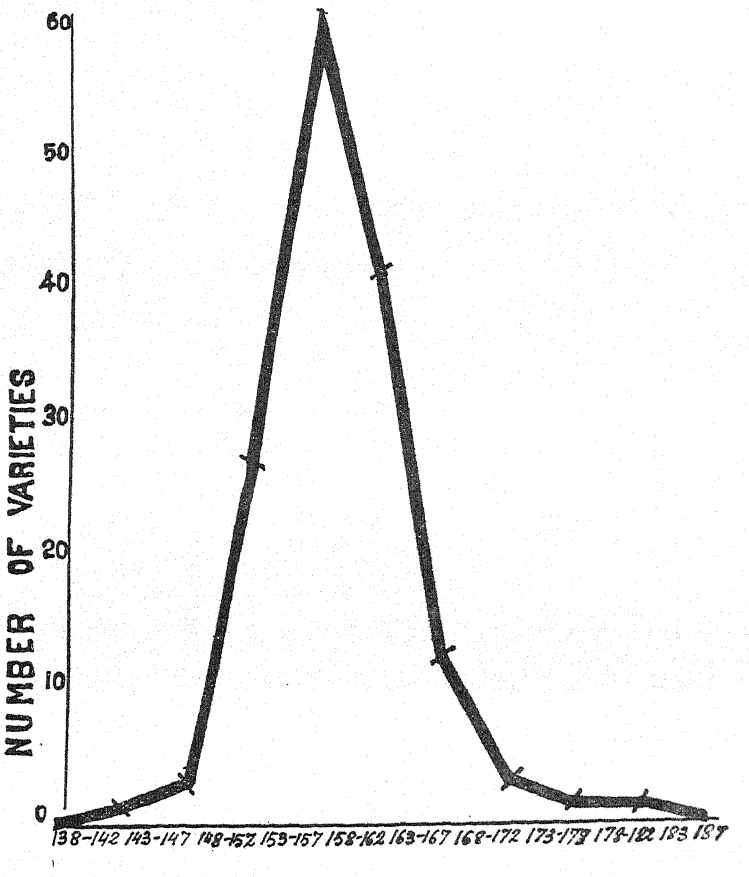


Fig. 4.—Curve showing the total duration (from sowing in seed-beds to ripening) of 857 types of transplanted *Aman* paddies. Average of 3 years, 1927-29.

Such a variation in the range of duration of the different varieties, therefore, offers opportunities to the cultivators for selecting the varieties particularly suitable for their own conditions. The selection of two or three such varieties suitable for two or three different situations also facilitates division of labour and prevents the overlapping of the different agricultural operations. There are large areas of intermediate situation in the province where double-cropping is also practised, *i.e.*, a broadcast *Aus* paddy is followed by a transplanted winter paddy. Varieties having short life periods are particularly suitable for such lands, because thereby, after the early harvest of the first crop, the cultivators are provided with a sufficient interval for preparing the land for the succeeding transplanted *Aman* crop.

The total duration of rice can be roughly divided into two parts, namely the vegetative period and reproductive period. In the case of transplanted paddies,

however, the vegetative period can be further subdivided into a nursery period, *i.e.*, the period from sowing to transplanting and a post-nursery period, *i.e.*, the period from transplanting to flowering and maturity.

In the case of highland broadcast *Aus* paddies the vegetative period remains more or less constant, whereas in the case of transplanted *Aman* paddies it is not so, and may be shortened or prolonged, depending on the time of sowing in the seed-bed, as well as the time of transplanting. But since the interval between flowering and maturity of any particular strain of *Aus* and *Aman* paddy is fairly constant, it is evident that the total duration of a paddy is more dependent on the vegetative period than on anything else. Therefore, in any system of classification, to determine the earliness or lateness of varieties, the grouping in the case of *Aus* paddies should be based on the total duration of the varieties, whereas in the case of transplanted *Aman* paddies, the same object can be attained by taking the flowering into consideration, since, as stated above, the actual date of flowering in the case of *Aman* paddies is very constant. Again, since the interval from flowering to maturity is fairly constant for any particular strain, this factor along with the actual date of flowering ultimately determines, with the greatest degree of accuracy, the earliness and lateness of the different strains.

For the purpose of classification the Bengal rices under study may be classified as follows with respect to earliness or lateness :—

Highland Aus :—

Based on the duration from sowing to maturity.

Early	up to 104 days.
Medium	105 to 119 days.
Late	120 days or over.

Transplanted Aman paddies :—

Based on the actual date of flowering.

Early	up to 14th October.
Medium	15th October to 24th October.
Late	25th October and later.

Summary of classificatory scheme.

The main characters on which the scheme of classification here adopted is based, have been described in the preceding pages. In the first place, varieties are divided into common (translucent) rices, glutinous rices, winged rices, clustered rices and

double-grained rices. Within these main varieties, further varietal classification is based on—

(1) Distribution of colour or absence of colour in the vegetative parts.
(2) Presence or absence of awns.
(3) Colour of the ripe husk.
(4) Colour of husked grain.
(5) Consistency of grain.
(6) Shape and size of grain.
(7) Quality of grain (oval fine, medium, coarse; oblong fine, medium, coarse, depending on measurements).

Within varieties as above defined, types are further grouped into highland *Aus* and transplant *Amans*, according to date of sowing and harvest, and each of these again into early, medium, and late, according to duration from sowing to harvest.

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Appendix I.

VARIETAL CLASSIFICATION OF EASTERN BENGAL *AUS* AND *AMAN* PADDIES.

I.—Common, translucent-grained paddies.

Variety No.						No. of types
			A.—Leaf-sheath, tip, stigma colourless—awnless.			
1	Glumes-yellow,	Grain-red,	Wholly translucent,	Oval,	Medium	a— 1
2	,,	,,	,,	,,	,,	c— 1
3	,,	,,	,,	,,	,,	d— 1
4	,,	,,	,,	,,	Coarse	b— 3
5	,,	,,	,,	,,	,,	c—20
6	,,	,,	,,	,,	,,	d—49
7	,,	,,	,,	,,	,,	c—25
8	,,	,,	,,	Oblong,	Fine.	c— 1
9	,,	,,	,,	,,	Medium	a— 1
10	,,	,,	,,	,,	,,	b— 4
11	,,	,,	,,	,,	,,	c— 2
12	,,	,,	,,	,,	,,	d— 2
13	,,	,,	,,	Oval,	Coarse	b— 6
14	,,	,,	,,	,,	,,	c—15
15	,,	,,	,,	,,	,,	d— 5
16	,,	,,	,,	,,	,,	e— 4
17	,,	,,	,,	,,	,,	i— 1
18	,,	,,	With abdominal white,	,,	,,	b— 3
19	,,	,,	,,	,,	,,	c— 9
20	,,	,,	,,	,,	,,	d—18
21	,,	,,	,,	,,	,,	e—13
22	,,	,,	,,	,,	,,	f— 1
23	,,	,,	,,	Oblong,	Coarse	c— 1
24	,,	,,	,,	,,	,,	d— 3
25	,,	,,	,,	,,	,,	f— 1
26	,,	Grain-amber,	Wholly translucent,	,,	Fine	c— 2
27	,,	,,	,,	,,	Coarse	c— 1
28	,,	,,	,,	,,	,,	d— 1
29	,,	,,	With abdominal white,	Oval	,,	e— 1
30	,,	,,	,,	,,	,,	f— 1
31	,,	Grains-white,	Wholly translucent,	Oval,	Fine	a— 7
32	,,	,,	,,	,,	,,	b—32
33	,,	,,	,,	,,	,,	c—14
34	,,	,,	,,	,,	Medium	a— 3

Appendix I—*contd.*

Variety No.						No. of types
35	Glumes-yellow,	Grains-white,	Wholly translucent,	Oval	Medium	b— 1
36	,,	,,	,,	,,	,,	c— 7
37	,,	,,	,,	,,	,,	d— 5
38	,,	,,	,,	,,	Coarse	a— 3
39	,,	,,	,,	,,	,,	b— 2
40	,,	,,	,,	,,	,,	c—15
41	,,	,,	,,	,,	,,	d—25
42	,,	,,	,,	,,	,,	e—21
43	,,	,,	,,	,,	,,	f— 1
44	,,	,,	,,	,,	,,	h— 2
45	,,	,,	,,	Oblong,	Fine	a— 2
46	,,	,,	,,	,,	,,	b— 2
47	,,	,,	,,	,,	,,	c— 9
48	,,	,,	,,	,,	,,	e— 1
49	,,	,,	,,	,,	,,	f— 1
50	,,	,,	,,	,,	Medium	a— 4
51	,,	,,	,,	,,	,,	b—18
52	,,	,,	,,	,,	,,	c— 6
53	,,	,,	,,	,,	,,	d— 4
54	,,	,,	,,	,,	,,	e— 3
55	,,	,,	,,	,,	,,	f— 1
56	,,	,,	,,	,,	Coarse	b— 5
57	,,	,,	,,	,,	,,	c—14
58	,,	,,	,,	,,	,,	d— 4
59	,,	,,	,,	,,	,,	f— 2
60	,,	,,	With abdominal white,	Oval,	Fine	b— 1
61	,,	,,	,,	,,	Coarse	a— 1
62	,,	,,	,,	,,	,,	c— 6
63	,,	,,	,,	,,	,,	d—11
64	,,	,,	,,	,,	,,	e— 7
65	,,	,,	,,	,,	,,	f— 1
66	,,	,,	,,	Oblong	,,	a— 1
67	,,	,,	,,	,,	,,	b— 2
68	,,	,,	,,	,,	,,	c— 1
69	Glumes-red,	Grains-red,	Wholly translucent,	Oval,	,,	d— 2
70	,,	,,	,,	,,	,,	e— 5
71	,,	,,	,,	,,	,,	f— 1
72	,,	,,	,,	,,	,,	g— 1
73	,,	,,	,,	Oblong,	Medium	c— 1
74	,,	,,	,,	,,	Coarse	c— 2
75	,,	,,	,,	,,	,,	d— 1
76	,,	,,	,,	,,	,,	e— 1
77	,,	,,	With abdominal white,	Oval,	,,	d— 1
78	,,	,,	,,	Oblong	,,	c— 1
79	Glumes-yellow,	Grains-white,	Wholly translucent,	Oval,	Fine	c— 2
80	,,	,,	,,	,,	Medium	a— 3
81	,,	,,	,,	,,	,,	c— 1

Appendix I—*contd.*

Variety No.						No. of types
82	Glumes-red,	Grains-white,	Wholly	translucent,	Oval, Coarse	a— 1
83	,,	,,	,,	,,	,,	b— 4
84	,,	,,	,,	,,	,,	c— 2
85	,,	,,	,,	,,	,,	d—14
86	,,	,,	,,	,,	,,	e— 7
87	,,	,,	,,	,,	,,	f— 4
88	,,	,,	,,	Oblong,	Fine	a— 1
89	,,	,,	,,	,,	,,	c— 2
90	,,	,,	,,	,,	,,	e— 1
91	,,	,,	,,	,,	Medium	b— 3
92	,,	,,	,,	,,	,,	c— 2
93	,,	,,	,,	,,	,,	e— 7
94	,,	,,	,,	,,	Coarse	b— 8
95	,,	,,	,,	,,	,,	c—20
96	,,	,,	,,	,,	,,	d— 4
97	,,	,,	,,	,,	,,	f— 1
98	,,	Grains-red,	With abdominal white,	Oval,	Medium	a— 1
99	,,	,,	,,	,,	Coarse	b— 2
100	,,	,,	,,	,,	,,	d— 2
101	,,	,,	,,	,,	,,	f— 3
102	,,	,,	,,	Oblong,	Medium	c— 1
103	,,	,,	,,	,,	Coarse	b— 1
104	,,	,,	,,	,,	,,	d— 1
105	Glumes-black,	Grains-red,	Wholly	translucent,	Oval, Coarse	c— 4
106	,,	,,	,,	,,	,,	d—16
107	,,	,,	,,	,,	,,	e—16
108	,,	,,	,,	,,	,,	g— 1
109	,,	,,	,,	Oblong,	Medium	c— 2
110	,,	,,	,,	,,	,,	d— 1
111	,,	,,	,,	,,	Coarse	e— 1
112	,,	,,	With abdominal white,	Oval,	,,	c— 5
113	,,	,,	,,	,,	,,	d—14
114	,,	,,	,,	,,	,,	e— 8
115	,,	Grains-white,	Wholly	translucent,	,, Fine	b— 3
116	,,	,,	,,	,,	Coarse	c— 1
117	,,	,,	,,	,,	,,	d— 4
118	,,	,,	,,	,,	,,	e— 1
119	,,	,,	,,	Oblong,	Medium	c— 1
120	,,	,,	,,	,,	Coarse	b— 3
121	,,	,,	,,	,,	,,	c— 6
122	,,	,,	With abdominal white,	Oval,	,,	d— 1
123	Glumes-mottled,	Grains-red,	Wholly	translucent,	,, ,,	c— 5
124	,,	,,	,,	,,	,,	d—29
125	,,	,,	,,	,,	,,	e—24

Appendix I—*contd.*

Variety No.						No. of types
126	Glumes-mottled,	Grains-red,	Wholly translucent,	Oval,	Coarse	f— 3
127	,,	,,	,,	Oblong,	Medium	a— 1
128	,,	,,	,,	,,	,,	c— 3
129	,,	,,	,,	,,	,,	d— 2
130	,,	,,	,,	,,	Coarse	b— 1
131	,,	,,	,,	,,	,,	c— 2
132	,,	,,	,,	,,	,,	d— 2
133	,,	,,	,,	,,	,,	e— 2
134	,,	,,	With abdominal white,	Oval,	,,	b— 1
135	,,	,,	,,	,,	,,	c—11
136	,,	,,	,,	,,	,,	d—18
137	,,	,,	,,	,,	,,	e—10
138	,,	,,	,,	,,	,,	f— 3
139	,,	,,	,,	,,	,,	g— 1
140	,,	Grains-amber,	Wholly translucent,	Oblong,	,,	c— 1
141	,,	,,	With abdominal white,	Oval,	,,	d— 2
142	,,	Grains-white,	Wholly translucent,	,,	Fine	b— 1
143	,,	,,	,,	,,	Coarse	b— 2
144	,,	,,	,,	,,	,,	c— 1
145	,,	,,	,,	,,	,,	d—10
146	,,	,,	,,	,,	,,	e— 2
147	,,	,,	,,	Oblong,	Medium	b— 1
148	,,	,,	,,	,,	,,	c— 3
149	,,	,,	,,	,,	,,	d— 2
150	,,	,,	,,	,,	Coarse	b— 4
151	,,	,,	,,	,,	,,	c—12
152	,,	,,	,,	,,	,,	d— 1
153	,,	,,	With abdominal white,	Oval,	,,	d— 2
154	,,	,,	,,	,,	,,	e— 2
155	Glumes-piebald,	Grains-red,	Wholly translucent,	Oval,	Coarse	c— 3
156	,,	,,	,,	,,	,,	d— 3
157	,,	,,	,,	,,	,,	e— 2
158	,,	,,	,,	Oblong,	,,	c— 1
159	,,	,,	With abdominal white,	Oval,	,,	d— 1
160	,,	,,	,,	,,	,,	e— 1
161	,,	Grains-white,	Wholly translucent,	,,	,,	c— 1
162	,,	,,	,,	,,	,,	d— 5
163	,,	,,	,,	,,	,,	e— 1
164	,,	,,	,,	Oblong,	Medium	b— 7
165	,,	,,	,,	,,	Coarse	b— 4
166	,,	,,	,,	,,	,,	c— 6
167	,,	,,	With abdominal white,	Oval,	,,	d— 2
168	,,	,,	,,	,,	,,	e— 1
169	,,	,,	,,	Oblong,	,,	e— 1

Appendix I—*contd.*

Variety No.						No. of types
	B. Leaf-sheath, tip, stigma colourless—awned.					
170	Glumes-yellow,	Grains-red,	Wholly translucent,	Oval,	Coarse	b— 1
171	,,	,,	,,	,,	,,	c— 5
172	,,	,,	,,	,,	,,	d—17
173	,,	,,	,,	,,	,,	e—22
174	,,	,,	,,	,,	,,	f— 7
175	,,	,,	,,	,,	,,	g— 2
176	,,	,,	,,	Oblong,	Fine	f— 1
177	,,	,,	,,	,,	Medium	c— 1
178	,,	,,	,,	,,	,,	d— 6
179	,,	,,	,,	,,	,,	e— 1
180	,,	,,	,,	,,	,,	f— 1
181	,,	,,	,,	,,	Coarse	c— 4
182	,,	,,	,,	,,	,,	d— 4
183	,,	,,	,,	,,	,,	e— 6
184	,,	,,	With abdominal white,	Oval,	,,	a— 1
185	,,	,,	,,	,,	,,	b— 4
186	,,	,,	,,	,,	,,	c— 2
187	,,	,,	,,	,,	,,	d—15
188	,,	,,	,,	,,	,,	e— 6
189	,,	,,	,,	,,	,,	f— 2
190	,,	,,	,,	,,	,,	g— 1
191	,,	,,	,,	Oblong,	Medium	f— 1
192	,,	,,	,,	,,	Coarse	d— 1
193	,,	,,	,,	,,	,,	e— 1
194	,,	,,	,,	,,	,,	f— 2
195	,,	Grains-amber,	Wholly translucent,	,,	Fine	d— 1
196	,,	,,	,,	,,	Medium	c— 1
197	,,	,,	,,	,,	,,	e— 1
198	,,	,,	,,	,,	Coarse	e— 1
199	,,	,,	,,	,,	,,	f— 1
200	,,	,,	With abdominal white,	Oval,	,,	e— 1
201	,,	Grains-white,	Wholly translucent,	,,	Medium	c— 1
202	,,	,,	,,	,,	,,	d— 3
203	,,	,,	,,	,,	Coarse	a— 2
204	,,	,,	,,	,,	,,	b— 2
205	,,	,,	,,	,,	,,	c— 7
206	,,	,,	,,	,,	,,	d— 6
207	,,	,,	,,	,,	,,	e— 1
208	,,	,,	,,	,,	Fine	b— 1
209	,,	,,	,,	,,	,,	c— 4
210	,,	,,	,,	,,	,,	d— 5
211	,,	,,	,,	,,	,,	e— 5
212	,,	,,	,,	,,	,,	g— 1
213	,,	,,	,,	,,	Medium	b— 1
214	,,	,,	,,	,,	,,	c— 6
215	,,	,,	,,	,,	,,	d— 3

Appendix I—*contd.*

Variety No.						No. of types
216	Glumes-yellow,	Grains-white,	Wholly translucent,	Oblong,	Medium	e— 1
217	,,	,,	,,	,,	,,	f— 1
218	,,	,,	,,	,,	,,	g— 2
219	,,	,,	,,	,,	Coarse	b— 1
220	,,	,,	,,	,,	,,	c— 3
221	,,	,,	,,	,,	,,	d— 2
222	,,	,,	,,	,,	,,	e— 3
223	,,	,,	With abdominal white,	Oval,	,,	c— 9
224	,,	,,	,,	,,	,,	d— 8
225	,,	,,	,,	,,	,,	e— 4
226	,,	,,	,,	Oblong,	Medium	d— 1
227	,,	,,	,,	,,	,,	f— 1
228	,,	,,	,,	,,	Coarse	d— 2
229	,,	,,	,,	,,	,,	c— 1
230	,,	,,	,,	,,	,,	f— 1
231	Glumes-red,	Grains-red,	Wholly translucent,	Oval,	,,	e— 1
232	,,	,,	,,	Oblong,	Medium	f— 2
233	,,	,,	,,	,,	Coarse	d— 1
234	,,	,,	,,	,,	,,	e— 5
235	,,	Grains-amber	,,	,,	,,	c— 1
236	,,	,,	With abdominal white,	,,	Medium	f— 1
237	,,	Grains-white,	Wholly translucent,	Oval,	Fine	b— 1
238	,,	,,	,,	,,	Coarse	d— 4
239	,,	,,	,,	,,	,,	e— 4
240	,,	,,	,,	Oblong,	Fine	c— 2
241	,,	,,	,,	,,	Medium	c— 1
242	,,	,,	,,	,,	Coarse	b— 2
243	,,	,,	,,	,,	,,	c— 5
244	,,	,,	With abdominal white,	Oval,	,,	c— 2
245	,,	,,	,,	,,	,,	d— 1
246	Glumes-black,	Grains-red,	Wholly translucent,	,,	,,	c— 3
247	,,	,,	,,	,,	,,	d— 8
248	,,	,,	,,	,,	,,	e—12
249	,,	,,	,,	,,	,,	f — 3
250	,,	,,	,,	Oblong,	,,	a— 1
251	,,	,,	,,	,,	,,	d— 1
252	,,	,,	With abdominal white,	Oval,	,,	c— 2
253	,,	,,	,,	,,	,,	d—14
254	,,	,,	,,	,,	,,	e— 6
255	,,	,,	,,	,,	,,	f— 1
256	,,	Grains-amber,	,,	,,	,,	d— 1
257	Glumes-yellow,	Grains-white,	Wholly translucent,	Oblong,	Fine	c— 1
258	,,	,,	,,	,,	Medium	c— 1
259	,,	,,	,,	,,	Coarse	c— 1

Appendix I—*contd.*

Variety No.						No. of types
260	Glumes-mottled,	Grains-red,	Wholly translucent,	Oval,	Coarse	c— 2
261	,,	,,	,,	,,	,,	d—16
262	,,	,,	,,	,,	,,	e—16
263	,,	,,	,,	,,	,,	f— 2
264	,,	,,	,,	Oblong,	Medium	b— 1
265	,,	,,	,,	,,	,,	c— 1
266	,,	,,	,,	,,	,,	d— 1
267	,,	,,	,,	,,	,,	f— 1
268	,,	,,	,,	,,	Coarse	b— 1
269	,,	,,	,,	,,	,,	c— 4
270	,,	,,	,,	,,	,,	d— 2
271	,,	,,	,,	,,	,,	e— 2
272	,,	,,	With abdominal white,	Oval,	,,	c— 2
273	,,	,,	,,	,,	,,	d—14
274	,,	,,	,,	,,	,,	e—11
275	,,	Grains-amber,	Wholly translucent,	,,	,,	d— 1
276	,,	,,	,,	,,	,,	e— 1
277	,,	Grains-white,	,,	,,	Fine	b— 1
278	,,	,,	,,	,,	Medium	d— 2
279	,,	,,	,,	,,	Coarse	d— 2
280	,,	,,	,,	,,	,,	e— 1
281	,,	,,	,,	,,	,,	f— 1
282	,,	,,	,,	,,	,,	g— 1
283	,,	,,	,,	,,	Medium	d— 3
284	,,	,,	,,	,,	,,	e— 2
285	,,	,,	,,	,,	Coarse	b— 2
286	,,	,,	,,	,,	,,	c— 8
287	,,	,,	With abdominal white,	,,	,,	d— 2
288	,,	,,	,,	,,	,,	e— 1
289	Glumes-piebald,	Grains-red,	Wholly translucent,	,,	,,	d— 2
290	,,	,,	With abdominal white.	,,	,,	d— 2
291	,,	Grains-white,	Wholly translucent,	,,	,,	c— 1
292	,,	,,	,,	,,	,,	d— 2
293	,,	,,	,,	,,	,,	e— 1
	C. Leaf-sheath, tip coloured, stigma colourless—awnless.					
294	Glumes-yellow,	Grains-red,	Wholly translucent,	Oval,	Medium	c— 1
295	,,	,,	,,	,,	Coarse.	c— 7
296	,,	,,	,,	,,	,,	d— 8
297	,,	,,	,,	,,	,,	e— 4
298	,,	,,	,,	Oblong,	,,	b— 1
299	,,	,,	With abdominal white,	Oval,	,,	b— 1
300	,,	,,	,,	,,	,,	c— 5
301	,,	,,	,,	,,	,,	d—10
302	,,	,,	,,	,,	,,	e— 1
303	,,	Grains-white,	Wholly translucent,	,,	Fine.	a— 2
304	,,	,,	,,	,,	,,	b—10

Appendix I—*contd.*

Variety No.						No. of types
305	Glumes-yellow,	Grains-white,	Wholly translucent,	Oval,	Fine	c— 2
306	,,	,,	,,	,,	Medium	a— 2
307	,,	,,	,,	,,	,,	b— 1
308	,,	,,	,,	,,	,,	c— 2
309	,,	,,	,,	,,	,,	d— 1
310	,,	,,	,,	,,	Coarse	c— 2
311	,,	,,	,,	,,		d— 4
312	,,	,,	,,	Oblong,	Fine,	c— 1
313	,,	,,	,,	,,	Medium	b— 1
314	,,	,,	,,	,,	,,	c— 1
315	,,	,,	,,	,,	,,	d— 1
316	,,	,,	,,	,,	,,	e— 1
317	,,	,,	,,	,,	,,	g— 1
318	,,	,,	With abdominal white,	Oval,	Fine	a— 1
319	,,	,,	,,	,,	Medium	a— 1
320	,,	,,	,,	,,	Coarse	c— 1
321	Glumes-red,	Grains-red,	Wholly translucent,	,,	,,	d— 1
322	,,	,,	,,	Oblong,	,,	b— 1
323	,,	,,	With abdominal white,	Oval,	,,	c— 1
324	,,	Grains-amber,	Wholly translucent,	,,	,,	c— 1
325	,,	,,	,,	Oblong,	Medium	b— 1
326	,,	,,	,,	,,	Coarse	b— 3
327	,,	Grains-white,	,,	Oval,	,,	d— 2
328	,,	,,	,,	,,	,,	c— 1
329	,,	,,	,,	,,	,,	f— 1
330	,,	,,	,,	,,	,,	g— 1
331	,,	,,	,,	Oblong,	,,	b— 1
332	,,	,,	,,	,,	,,	c— 2
333	,,	,,	With abdominal white,	Oval	,,	g— 1
334	,,	,,	,,	Oblong	,,	c— 1
335	Glumes-black,	Grains-red,	Wholly translucent,	Oval,	,,	c— 1
336	,,	,,	,,	,,	,,	d— 3
337	,,	,,	,,	Oblong,	,,	c— 1
338	,,	Grains-amber,	,,	Oval,	Fine	c— 1
339	,,	,,	,,	,,	Medium	c— 1
340	,,	,,	,,	,,	,,	d— 1
341	,,	,,	,,	,,	Coarse	c— 1
342	,,	Grains-white,	,,	,,	Fine	a— 1
343	,,	,,	,,	,,	,,	b— 8
344	,,	,,	,,	,,	,,	c— 3
345	,,	,,	,,	,,	Medium	a— 1
346	,,	,,	,,	,,	Coarse	g— 1
347	,,	,,	,,	Oblong,	Fine	b— 1
348	,,	,,	,,	,,	Medium	a— 1
349	,,	,,	,,	,,	,,	c— 1
350	,,	,,	,,	,,	,,	d— 1
351	,,	,,	,,	,,	Coarse	c— 1

Appendix I—*contd.*

Variety No.						No. of types
352	Glumes-black,	Grains-white,	With abdominal white,	Oval,	Coarse	c— 1
353	Glumes-mottled,	Grains-red,	Wholly translucent,	,,	,,	c— 1
354	,,	,,	,,	,,	,,	d— 1
355	,,	,,	,,	Oblong	,,	a— 1
356	,,	,,	With abdominal white,	Oval,	,,	d— 2
357	,,	Grains-white,	Wholly translucent,	,,	Fine	c— 1
358	,,	,,	,,	Oblong,	Coarse	c— 1
359	Glumes-piebald,	,,	,,	Oval,	Fine	a— 2

D. Leaf-sheath, tip coloured, stigma colourless—awned.

360	Glumes-yellow,	Grains-red,	Wholly translucent,	Oval,	Coarse	d— 3
361	,,	,,	,,	,,	,,	e— 4
362	,,	,,	With abdominal white	,,	,,	c— 1
363	,,	,,	,,	,,	,,	d— 7
364	,,	,,	,,	,,	,,	e— 2
365	,,	Grains-amber,	Wholly translucent,	,,	,,	c— 1
366	,,	Grains-white,	,,	Oblong,	Medium.	c— 2
367	,,	,,	With abdominal white,	Oval,	Coarse	b— 1
368	,,	,,	,,	,,	,,	c— 1
369	,,	,,	,,	,,	,,	d— 1
370	Glumes-red,	Grains-red,	Wholly translucent,	,,	,,	d— 1
371	,,	,,	With abdominal white,	,,	,,	d— 2
372	,,	Grains-amber,	Wholly translucent,	,,	,,	e— 1
373	,,	,,	With abdominal white,	,,	,,	d— 2
374	,,	,,	,,	,,	,,	e— 2
375	,,	Grains-white,	Wholly translucent,	,,	Fine	c— 1
376	,,	,,	,,	,,	Coarse	a— 1
377	,,	,,	,,	,,	,,	b— 3
378	,,	,,	,,	,,	,,	d— 2
379	,,	,,	,,	Oblong,	Fine	c— 1
380	,,	,,	,,	,,	Medium	b— 2
381	,,	,,	,,	,,	Coarse	c— 2
382	,,	,,	,,	,,	,,	d— 1
383	Glumes-black,	Grains-red,	Wholly translucent,	Oval,	,,	d— 2
384	,,	,,	With abdominal white	,,	,,	d— 1
385	,,	,,	,,	Oblong,	Medium	c— 1
386	,,	Grains-white,	Wholly translucent,	Oval,	Fine	b— 1
387	,,	,,	With abdominal white,	,,	Coarse	d— 2
388	Glumes-mottled,	Grains-red,	Wholly translucent,	,,	,,	d— 1
389	,,	,,	,,	,,	,,	f— 1
390	,,	,,	With abdominal white,	,,	,,	d— 5
391	,,	,,	,,	,,	,,	e—10
392	,,	Grains-white,	Wholly translucent	,,	,,	d— 1

E. Leaf-sheath, tip and stigma coloured—awnless.

393	Glumes-yellow,	Grains-red,	Wholly translucent,	Oval,	Coarse	a— 1
394	,,	,,	,,	,,	,,	b— 1

Appendix I—*contd.*

Variety No.		No. of types
395	Glumes-yellow, Grains-red, Wholly translucent Oval, Course	c—11
396	,, ,, ,, ,, ,,	d—23
397	,, ,, ,, ,, ,,	e— 3
398	,, ,, ,, ,, ,,	f— 1
399	,, ,, ,, Oblong ,,	c— 2
400	,, ,, With abdominal white, Oval, ,,	c— 7
401	,, ,, ,, ,, ,,	d—14
402	,, ,, ,, ,, ,,	e— 4
403	,, ,, ,, Oblong, ,,	c— 1
404	,, ,, ,, ,, ,,	d— 1
431	Glumes-red, Grains-white, Wholly translucent, Oval, Coarse	c— 1
432	,, ,, ,, ,, ,,	d— 1
433	,, ,, ,, ,, ,,	e— 1
434	,, ,, With abdominal white ,, ,,	c— 1
535	,, ,, ,, ,, ,,	f— 1
436	,, ,, Wholly translucent, Oblong, Medium	a— 1
437	,, ,, ,, ,, ,,	b— 2
438	,, ,, ,, ,, Coarse	b— 2
439	,, ,, ,, ,, ,,	c— 1
440	Glumes-black, Grains-red, ,, Oval, ,,	c— 9
441	,, ,, ,, ,, ,,	d— 8
442	,, ,, ,, ,, ,,	e— 3
443	,, ,, With abdominal white. ,, ,,	c— 2
444	,, ,, ,, ,, ,,	d— 4
445	,, ,, ,, ,, ,,	e— 3
446	,, ,, ,, ,, ,,	f— 1
447	,, Grains-amber, ,, Oblong ,,	d— 1
448	,, Grains-white, Wholly translucent, Oval, ,,	d— 1
449	,, ,, With abdominal white, ,, ,,	d— 2
450	Glumes-mottled, Grains-red, Wholly translucent, ,, ,,	c— 2
451	,, ,, ,, ,, ,,	d— 2
452	,, ,, ,, ,, ,,	e— 9
453	,, ,, ,, Oblong, Medium	c— 1
454	,, ,, ,, ,, ,,	d— 2
455	,, ,, ,, ,, Coarse	c— 2
456	,, ,, With abdominal white, Oval, ,,	b— 1
457	,, ,, ,, ,, ,,	c— 4
458	,, ,, ,, ,, ,,	d—11
459	,, ,, ,, ,, ,,	e— 2
460	,, ,, ,, Oblong, ,,	c— 1
461	,, Grains-amber, Wholly translucent, Oval, ,,	e— 1
462	,, Grains-white, ,, ,, ,,	b— 1
463	,, ,, ,, ,, ,,	c— 1
464	,, ,, ,, ,, ,,	d— 1
465	Glumes-piebald, Grains-red, ,, ,, ,,	d— 1

Appendix I—*contd.*

Variety No.		No. of types
	F. Leaf-sheath, tip, stigma coloured—awned.	
466	Glumes-yellow, Grains-red, Wholly translucent, Oval, Coarse	c—12
467	,, ,, ,, ,, ,,	d—10
468	,, ,, ,, ,, ,,	e— 5
469	,, ,, ,, Oblong, ,,	b— 1
470	,, ,, ,, ,, ,,	d— 1
471	,, ,, With abdominal white, Oval, ,,	c— 2
472	,, ,, ,, ,, ,,	d— 3
473	,, ,, ,, ,, ,,	e— 2
474	,, ,, ,, Oblong, Medium	c— 1
475	,, Grains-amber, Wholly translucent, Oval, Coarse	d— 1
476	,, ,, ,, ,, ,,	e— 1
477	,, Grains-white, ,, Oblong, Medium	c— 1
478	,, ,, ,, ,, ,,	d— 1
479	,, ,, ,, ,, Coarse	f— 1
480	,, ,, With abdominal white, Oval, ,,	c— 1
481	,, Grains-red, Wholly translucent, ,, ,,	d— 2
482	,, ,, ,, Oblong, ,,	c— 4
483	,, ,, With abdominal white, Oval, ,,	e— 1
484	,, Grains-amber, Wholly translucent, ,, Medium	c— 1
485	,, ,, ,, ,, Coarse	d— 2
486	,, ,, ,, Oblong, Medium	c— 1
487	,, ,, ,, ,, ,,	e— 1
488	Glumes-red, ,, Wholly translucent, ,, ,, Coarse	c— 1
489	,, Grains-white ,, Oval, ,,	d— 2
490	,, ,, ,, Oblong, Medium	a— 1
491	,, ,, ,, ,, ,,	b— 2
492	,, ,, ,, ,, ,,	e— 1
493	Glumes-red, Grains-white, Wholly translucent, Oblong, Coarse	c— 1
494	Glumes-black, Grains-red, ,, Oval, ,,	c— 2
495	,, ,, ,, ,, ,,	d— 3
496	,, ,, ,, ,, ,,	e— 1
497	,, ,, With abdominal white, ,, ,,	e— 2
498	,, Grains-amber, Wholly translucent, Oblong ,,	c— 1
499	Glumes-mottled, Grains-red, ,, Oval, ,,	a— 1
500	,, ,, ,, ,, ,,	b— 1
501	,, ,, ,, ,, ,,	e— 3
502	,, ,, ,, ,, ,,	d— 9
503	,, ,, ,, ,, ,,	e— 2
504	,, ,, ,, Oblong, Medium	d— 2
505	,, ,, With abdominal white, Oval, Coarse	c— 3
506	,, ,, ,, ,, ,,	d— 3
507	,, ,, ,, ,, ,,	e— 3

Appendix I—*contd.*

II.—Glutinous paddies.

Variety No.						No. of types
			A. Leaf-sheath, tip, stigma colourless—awnless.			
508	Glumes-red,	Grains-red,	Glutinous,	Oblong,	Coarse	c— 1
509	,,	Grains-amber,	,,	,,	,,	b— 1
510	,,	Grains-white,	,,	Oval	,,	c— 1
511	,,	,,	,,	Oblong	,,	d— 1
			B. Leaf-sheath, tip, stigma colourless—awned.			
512	Glumes-yellow,	Grains-red,	Glutinous,	Oblong,	Medium	e— 1
513	,,	Grains-white,	,,	Oval,	Coarse	b— 1
514	,,	,,	,,	,,	,,	c— 2
515	Glumes-black,	Grains-amber,	,,	Oblong	,,	d— 1
			C. Leaf-sheath, tip coloured, stigma colourless—awnless.			
516	Glumes-black,	Grains-red,	Glutinous,	Oval,	Coarse	e— 1
517	,,	,,	,,	Oblong,	,,	b— 1
			D. Leaf-sheath, tip and stigma coloured—awned.			
518	Glumes-yellow,	Grains-red,	Glutinous,	Oblong,	Coarse	e— 1
519	,,	Grains-white,	,,	,,	,,	e— 1
520	Glumes-mottled,	Grains-red,	,,	,,	Medium	e— 1

III.—Pankhiraj paddies (Winged paddies).

			A. Leaf-sheath, tip and stigma colourless—awnless.			
521	Glumes-yellow,	Grains-white,	Wholly translucent,	Oval,	Coarse	e— 1
522	,,	,,	,,	,,	,,	f— 1
523	Glumes-black,	Grains-red,	,,	,,	,,	d— 2
524	,,	,,	,,	,,	,,	e— 1
525	,,	,,	,,	,,	,,	f— 1
526	,,	Grains-white,	,,	Oblong	,,	e— 2
			B. Leaf-sheath, tip and stigma colourless—awned.			
527	Glumes-black,	Grains-red,	Wholly translucent,	Oval,	Coarse	e— 1
528	,,	,,	,,	,,	,,	f— 1
			C. Leaf sheath, tip coloured, stigma colourless—awnless.			
529	Glumes-mottled,	Grains-red,	Wholly translucent,	Oval,	Coarse	d— 1
530	,,	,,	With abdominal white	,,	,,	e— 1
			D. Leaf-sheath, tip and stigma coloured—awnless.			
531	Glumes-yellow,	Grains-red,	Wholly translucent,	Oval,	Coarse	e— 1
532	Glumes-black,	,,	,,	,,	,,	d— 2
533	,,	,,	,,	,,	,,	e— 2
534	,,	,,	With abdominal white,	,,	,,	d— 1

Appendix I—*concld.*

IV.—Clustered paddies.

Variety No.		No. of types
	A. Leaf-sheath, tip and stigma colourless—awnless.	
535	Glumes-yellow, Grains-red, With abdominal white, Oval, Coarse	c— 1
536	,, Grains-white, ,, ,, ,, ...	b— 1
537	,, ,, ,, ,, ,, ...	e— 1
538	Glumes-black, Grains-red, Wholly translucent, ,, ,, ...	g— 1
539	Glumes-piebald, ,, ,, ,, ,, ...	d— 1

V.—Double rice paddy.

	A. Leaf-sheath, tip and stigma coloured—awnless.	
540	Glumes-yellow, Grains-white, Wholly translucent, Oval, Coarse	e— 1

Appendix II.

TYPE CLASSIFICATION OF EASTERN BENGAL PADDIES.

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
1	1	Aus	1	1	..	..
		Aman	0	..	..	..
2	1	Aus	1	..	1	..
		Aman	0	..	..	..
3	1	Aus	1	..	1	..
		Aman	0	..	..	..
4	3	Aus	3	1	2	..
		Aman	0	..	..	..
5	20	Aus	18	4	11	3
		Aman	2	2	..	..
6	49	Aus	38	14	23	1
		Aman	11	..	11	..
7	25	Aus	16	13	3	..
		Aman	9	..	9	..
8	1	Aus	1	..	..	1
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
9	1	Aus	0	..	..	..
		Aman	1	1	..	..
10	4	Aus	0	..	..	..
		Aman	4	..	4	..
11	2	Aus	2	..	2	..
		Aman	0	..	..	..
12	2	Aus	0	..	..	..
		Aman	2	..	2	..
13	6	Aus	3	..	3	..
		Aman	3	..	3	..
14	15	Aus	8	2	6	..
		Aman	7	..	7	..
15	5	Aus	0	..	..	..
		Aman	5	1	4	..
16	4	Aus	0	..	..	..
		Aman	4	1	3	..
17	1	Aus	0	..	..	..
		Aman	1	..	1	..
18	3	Aus	3	..	3	..
		Aman	0	..	..	..
19	9	Aus	9	1	7	1
		Aman	0	..	..	..
20	18	Aus	17	3	14	..
		Aman	1	..	1	..
21	13	Aus	8	1	7	..
		Aman	5	2	3	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
22	1	Aus	0	..	..	..
		Aman	1	..	1	..
23	1	Aus	0	..	..	..
		Aman	1	..	1	..
24	3	Aus	2	..	2	..
		Aman	1	..	1	..
25	1	Aus	1	..	1	..
		Aman	0	..	..	..
26	2	Aus	2	..	2	..
		Aman	0	..	..	..
27	1	Aus	1	..	1	..
		Aman	0	..	..	..
28	1	Aus	1	..	1	..
		Aman	0	..	..	..
29	1	Aus	1	..	1	..
		Aman	0	..	..	..
30	1	Aus	0	..	..	..
		Aman	1	..	1	..
31	7	Aus	0	..	..	..
		Aman	7	..	7	..
32	32	Aus	1	..	1	..
		Aman	31	2	29	..
33	14	Aus	0	..	..	..
		Aman	14	3	11	..
34	3	Aus	0	..	..	..
		Aman	3	..	3	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
35	1	Aus	0	..	..	..
		Aman	1	..	1	..
36	7	Aus	4	..	4	..
		Aman	3	..	3	..
37	5	Aus	1	..	1	..
		Aman	4	..	4	..
38	3	Aus	0	..	..	..
		Aman	3	..	3	..
39	2	Aus	0	..	..	..
		Aman	2	..	2	..
40	15	Aus	7	..	7	..
		Aman	8	..	8	..
41	25	Aus	7	..	6	1
		Aman	18	1	16	1
42	21	Aus	2	..	1	1
		Aman	19	2	15	2
43	1	Aus	0	..	..	..
		Aman	1	..	1	..
44	2	Aus	0	..	..	..
		Aman	2	..	2	..
45	2	Aus	0	..	..	..
		Aman	2	..	2	..
46	2	Aus	0	..	..	..
		Aman	2	..	2	..
47	9	Aus	0	..	..	..
		Aman	9	1	8	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
48	1	Aus	1	..	1	..
		Aman	0	..	..	..
49	1	Aus	0	..	..	..
		Aman	1	..	1	..
50	4	Aus	0	..	..	..
		Aman	4	..	4	..
51	18	Aus	0	..	..	..
		Aman	18	4	14	..
52	6	Aus	1	..	1	..
		Aman	5	..	4	1
53	4	Aus	2	..	2	..
		Aman	2	..	2	..
54	3	Aus	0	..	..	..
		Aman	3	..	3	..
55	1	Aus	0	..	..	..
		Aman	1	..	1	..
56	5	Aus	3	..	3	..
		Aman	2	1	1	..
57	14	Aus	4	..	4	..
		Aman	10	1	9	..
58	4	Aus	0	..	..	..
		Aman	4	1	3	..
59	2	Aus	0	..	..	..
		Aman	2	..	2	..
60	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
61	1	Aus	0	..	..	..
		Aman	1	..	1	..
62	6	Aus	3	..	2	1
		Aman	3	..	2	1
63	11	Aus	3	..	3	..
		Aman	8	..	7	1
64	7	Aus	0	..	..	..
		Aman	7	..	4	3
65	1	Aus	0	..	..	..
		Aman	1	..	..	1
66	1	Aus	1	..	1	..
		Aman	0	..	..	..
67	2	Aus	2	1	1	..
		Aman	0	..	..	..
68	1	Aus	0	..	..	..
		Aman	1	..	1	..
69	2	Aus	0	..	..	..
		Aman	2	..	2	..
70	5	Aus	1	..	..	1
		Aman	4	..	4	..
71	1	Aus	0	..	..	..
		Aman	1	..	1	..
72	1	Aus	0	..	..	..
		Aman	1	..	1	..
73	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
74	2	Aus	0	..	..	..
		Aman	2	..	2	..
75	1	Aus	0	..	..	..
		Aman	1	..	1	..
76	1	Aus	1	..	1	..
		Aman	0	..	..	..
77	1	Aus	1	..	1	..
		Aman	0	..	..	..
78	1	Aus	1	..	1	..
		Aman	0	..	..	..
79	2	Aus	0	..	..	..
		Aman	2	1	1	..
80	3	Aus	0	..	..	..
		Aman	3	..	3	..
81	1	Aus	0	..	..	..
		Aman	1	..	1	..
82	1	Aus	0	..	..	..
		Aman	1	..	1	..
83	4	Aus	0	..	..	..
		Aman	4	..	4	..
84	2	Aus	0	..	..	..
		Aman	2	..	2	..
85	14	Aus	2	..	2	..
		Aman	12	..	12	..
86	7	Aus	0	..	..	..
		Aman	7	1	6	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
87	4	Aus	0	..	..	..
		Aman	4	1	3	..
88	1	Aus	0	..	..	..
		Aman	1	..	1	..
89	2	Aus	0	..	..	..
		Aman	2	..	2	..
90	1	Aus	1	..	1	..
		Aman	0	..	..	..
91	3	Aus	0	..	..	..
		Aman	3	2	1	..
92	2	Aus	0	..	..	..
		Aman	2	..	2	..
93	2	Aus	0	..	..	..
		Aman	2	..	2	..
94	8	Aus	0	..	..	..
		Aman	8	3	5	..
95	20	Aus	0	..	..	..
		Aman	20	2	18	..
96	4	Aus	0	..	..	..
		Aman	4	..	4	..
97	1	Aus	0	..	..	..
		Aman	1	..	1	..
98	1	Aus	0	..	..	..
		Aman	1	..	1	..
99	2	Aus	0	..	..	..
		Aman	2	1	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
100	2	Aus	0	..	..	..
		Aman	2	..	2	..
101	3	Aus	0	..	..	..
		Aman	3	..	3	..
102	1	Aus	0	..	..	..
		Aman	1	..	1	..
103	1	Aus	0	..	..	..
		Aman	1	..	1	..
104	1	Aus	0	..	..	..
		Aman	1	..	1	..
105	4	Aus	4	3	1	..
		Aman	0	..	..	..
106	16	Aus	11	8	3	..
		Aman	5	..	5	..
107	16	Aus	9	6	3	..
		Aman	7	1	6	..
108	1	Aus	0	..	..	..
		Aman	1	..	1	..
109	2	Aus	0	..	..	..
		Aman	2	..	2	..
110	1	Aus	0	..	..	..
		Aman	1	..	1	..
111	1	Aus	0	..	..	..
		Aman	1	..	1	..
112	5	Aus	5	4	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
113	14	Aus	14	9	5	..
		Aman	0	..	..	..
114	8	Aus	6	4	2	..
		Aman	2	..	2	..
115	3	Aus	0	..	..	..
		Aman	3	..	3	..
116	1	Aus	0	..	..	..
		Aman	1	..	1	..
117	4	Aus	0	..	..	..
		Aman	4	..	4	..
118	1	Aus	0	..	..	..
		Aman	1	..	1	..
119	1	Aus	0	..	..	..
		Aman	1	..	1	..
120	3	Aus	0	..	..	..
		Aman	3	1	2	..
121	6	Aus	0	..	..	..
		Aman	6	..	6	..
122	1	Aus	0	..	..	..
		Aman	1	..	1	..
123	5	Aus	3	1	2	..
		Aman	2	..	2	..
124	29	Aus	22	5	17	..
		Aman	7	3	4	..
125	24	Aus	18	8	10	..
		Aman	6	1	5	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
126	3	Aus	1	1	..	..
		Aman	2	..	2	..
127	1	Aus	0	..	..	..
		Aman	1	..	1	..
128	3	Aus	1	1	..	..
		Aman	2	..	2	..
129	2	Aus	2	..	2	..
		Aman	0	..	..	..
130	1	Aus	1	..	..	1
		Aman	0	..	..	..
131	2	Aus	1	1	..	..
		Aman	1	..	1	..
132	2	Aus	2	..	2	..
		Aman	0	..	..	..
133	2	Aus	1	..	1	..
		Aman	1	..	1	..
134	1	Aus	1	..	1	..
		Aman	0	..	..	..
135	12	Aus	12	4	7	1
		Aman	0	..	..	..
136	18	Aus	16	2	14	..
		Aman	2	..	2	..
137	10	Aus	6	1	4	1
		Aman	4	1	3	..
138	3	Aus	0	..	..	..
		Aman	3	..	3	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
139	1	Aus	0	..	..	..
		Aman	1	..	1	..
140	1	Aus	0	..	..	..
		Aman	1	..	1	..
141	2	Aus	1	..	1	..
		Aman	1	..	..	1
142	1	Aus	0	..	..	..
		Aman	1	..	1	..
143	2	Aus	0	..	..	..
		Aman	2	..	2	..
144	1	Aus	0	..	..	..
		Aman	1	..	1	..
145	10	Aus	0	..	..	..
		Aman	10	..	10	..
146	2	Aus	0	..	..	..
		Aman	2	..	2	..
147	1	Aus	0	..	..	..
		Aman	1	..	1	..
148	3	Aus	0	..	..	..
		Aman	3	..	3	..
149	2	Aus	0	..	..	..
		Aman	2	..	2	..
150	4	Aus	0	..	..	..
		Aman	4	..	4	..
151	12	Aus	0	..	..	..
		Aman	12	2	10	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
152	1	Aus	0	..	..	..
		Aman	1	..	1	..
153	2	Aus	0	..	..	..
		Aman	2	..	2	..
154	2	Aus	0	..	..	—
		Aman	2	..	2	.
155	3	Aus	3	..	3	..
		Aman	0	..	..	..
156	3	Aus	3	..	2	1
		Aman	0	..	..	..
157	2	Aus	2	..	2	..
		Aman	0	..	..	..
158	1	Aus	1	..	1	..
		Aman	0	..	..	..
159	1	Aus	1	..	..	1
		Aman	0	..	..	..
160	1	Aus	1	.	1	..
		Aman	0	..	..	..
161	1	Aus	1	..	1	..
		Aman	0	..	..	..
162	5	Aus	3	..	3	..
		Aman	2	..	2	..
163	1	Aus	0	..	..	..
		Aman	1	..	1	..
164	7	Aus	1	..	1	..
		Aman	6	..	6	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
165	4	Aus	0	..	..	..
		Aman	4	..	4	..
166	6	Aus	0	..	..	..
		Aman	6	..	6	..
167	2	Aus	2	..	2	..
		Aman	0	..	..	..
168	1	Aus	1	..	1	..
		Aman	0	..	..	..
169	1	Aus	0	..	..	..
		Aman	1	..	1	..
170	1	Aus	1	1	..	..
		Aman	0	..	..	..
171	5	Aus	5	..	5	..
		Aman	0	..	..	..
172	17	Aus	12	3	9	..
		Aman	5	..	5	..
173	22	Aus	13	5	7	1
		Aman	9	1	8	..
174	7	Aus	4	2	1	1
		Aman	3	..	3	..
175	2	Aus	2	..	2	..
		Aman	0	..	..	..
176	1	Aus	0	..	..	..
		Aman	1	..	1	..
177	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
178	6	Aus	3	..	3	..
		Aman	3	..	3	..
179	1	Aus	1	..	1	..
		Aman	0	..	..	..
180	1	Aus	1	..	1	..
		Aman	0	..	..	..
181	4	Aus	1	..	1	..
		Aman	3	..	3	..
182	4	Aus	2	..	2	..
		Aman	2	..	2	..
183	6	Aus	3	1	2	..
		Aman	3	..	3	..
184	1	Aus	1	1	..	..
		Aman	0	..	..	..
185	4	Aus	4	2	2	..
		Aman	0	..	..	..
186	2	Aus	2	..	2	..
		Aman	0	..	..	..
187	15	Aus	12	2	10	..
		Aman	3	1	2	..
188	6	Aus	6	3	3	..
		Aman	0	..	..	..
189	2	Aus	2	1	1	..
		Aman	0	..	..	..
190	1	Aus	1	1	..	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
191	1	Aus	1	..	1	..
		Aman	0	..	..	..
192	1	Aus	1	..	1	..
		Aman	0	..	..	..
193	1	Aus	0	..	..	..
		Aman	1	..	1	..
194	2	Aus	2	..	2	..
		Aman	0	..	..	..
195	1	Aus	1	..	1	..
		Aman	0	..	..	..
196	1	Aus	0	..	..	..
		Aman	1	..	1	..
197	1	Aus	0	..	..	..
		Aman	1	..	1	..
198	1	Aus	0	..	..	..
		Aman	1	..	1	..
199	1	Aus	0	..	..	..
		Aman	1	..	1	..
200	1	Aus	1	..	..	1
		Aman	0	..	..	..
201	1	Aus	0	..	..	..
		Aman	1	..	1	..
202	3	Aus	1	..	1	..
		Aman	2	..	2	..
203	2	Aus	0	..	..	..
		Aman	2	..	2	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
204	2	Aus	0	..	..	..
		Aman	2	..	2	..
205	7	Aus	1	..	1	..
		Aman	6	..	6	..
206	6	Aus	0	..	..	..
		Aman	6	..	6	..
207	1	Aus	1	..	..	1
		Aman	0	..	..	..
208	1	Aus	0	..	..	..
		Aman	1	..	1	..
209	4	Aus	1	..	1	..
		Aman	3	..	3	..
210	5	Aus	1	..	1	..
		Aman	4	..	4	..
211	5	Aus	3	..	3	..
		Aman	2	..	2	..
212	1	Aus	0	..	..	..
		Aman	1	..	1	..
213	1	Aus	0	..	..	..
		Aman	1	..	1	..
214	6	Aus	1	..	1	..
		Aman	5	1	4	..
215	3	Aus	0	..	..	..
		Aman	3	..	3	..
216	1	Aus	0	..	..	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
217	1	Aus	0	..	..	..
		Aman	1	..	1	..
218	2	Aus	0	..	..	..
		Aman	2	..	2	..
219	1	Aus	0	..	..	..
		Aman	1	..	1	..
220	3	Aus	0	..	..	..
		Aman	3	..	3	..
221	2	Aus	0	..	..	..
		Aman	2	..	2	..
222	3	Aus	0	..	..	..
		Aman	3	..	3	..
223	9	Aus	3	..	3	..
		Aman	6	..	6	..
224	8	Aus	3	..	..	..
		Aman	5	..	5	3
225	4	Aus	0	..	..	..
		Aman	4	..	4	..
226	1	Aus	0	..	..	..
		Aman	1	..	1	..
227	1	Aus	1	..	1	..
		Aman	0	..	..	..
228	2	Aus	2	..	2	..
		Aman	0	..	..	..
229	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
230	1	Aus	1	..	1	..
		Aman	0	..	..	..
231	1	Aus	0	..	..	..
		Aman	1	..	1	..
232	2	Aus	0	..	..	..
		Aman	2	1	1	..
233	1	Aus	0	..	..	..
		Aman	1	..	1	..
234	5	Aus	0	..	..	..
		Aman	5	..	5	..
235	1	Aus	1	..	1	..
		Aman	0	..	..	..
236	1	Aus	1	..	1	..
		Aman	0	..	..	..
237	1	Aus	0	..	..	..
		Aman	1	..	1	..
238	4	Aus	0	..	..	..
		Aman	4	1	3	..
239	4	Aus	0	..	..	..
		Aman	4	..	4	..
240	2	Aus	2	..	1	1
		Aman	0	..	..	..
241	1	Aus	1	..	..	1
		Aman	0	..	..	..
242	2	Aus	0	..	..	..
		Aman	2	..	2	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
243	5	Aus	0	..	..	..
		Aman	5	1	4	..
244	2	Aus	0	..	..	..
		Aman	2	..	2	..
245	1	Aus	0	..	..	..
		Aman	1	..	1	..
246	3	Aus	3	1	..	..
		Aman	0	..	..	..
247	8	Aus	8	6	2	..
		Aman	0	..	..	..
248	12	Aus	12	9	3	..
		Aman	0	..	..	..
249	3	Aus	2	1	1	..
		Aman	1	..	1	..
250	1	Aus	1	1	..	..
		Aman	0	..	..	..
251	1	Aus	0	..	..	..
		Aman	1	..	1	..
252	2	Aus	2	1	1	..
		Aman	0	..	..	..
253	14	Aus	13	7	6	..
		Aman	1	..	1	..
254	6	Aus	5	5	..	..
		Aman	1	..	1	..
255	1	Aus	1	1	..	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
256	1	Aus	1	..	1	..
		Aman	0	..	..	..
257	1	Aus	0	..	..	..
		Aman	1	..	1	..
258	1	Aus	0	..	..	..
		Aman	1	..	1	..
259	1	Aus	0	..	..	..
		Aman	1	..	1	..
260	2	Aus	1	1	..	..
		Aman	1	1	..	..
261	16	Aus	11	4	5	2
		Aman	5	1	4	..
262	16	Aus	7	5	2	..
		Aman	9	2	7	..
263	2	Aus	1	..	1	..
		Aman	1	..	1	..
264	1	Aus	0	..	..	..
		Aman	1	..	1	..
265	1	Aus	0	..	..	..
		Aman	1	..	1	..
266	1	Aus	0	..	..	..
		Aman	1	..	1	..
267	1	Aus	1	..	1	..
		Aman	0	..	..	..
268	1	Aus	0	..	..	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
269	4	Aus	0	..	..	..
		Aman	4	..	4	..
270	2	Aus	1	..	..	1
		Aman	1	..	1	..
271	2	Aus	2	..	2	..
		Aman	0	..	..	..
272	2	Aus	2	..	2	..
		Aman	0	..	..	..
273	14	Aus	13	3	10	..
		Aman	1	..	1	..
274	11	Aus	9	2	7	..
		Aman	2	..	2	..
275	1	Aus	0	..	..	..
		Aman	1	..	1	..
276	1	Aus	0	..	..	..
		Aman	1	..	1	..
277	1	Aus	0	..	..	..
		Aman	1	..	1	..
278	2	Aus	0	..	..	..
		Aman	2	1	1	..
279	2	Aus	0	..	..	..
		Aman	2	..	2	..
280	1	Aus	0	..	..	..
		Aman	1	..	1	..
281	1	Aus	0	..	..	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
282	1	Aus	0	..	..	..
		Aman	1	..	1	..
283	3	Aus	0	..	..	..
		Aman	3	..	3	..
284	2	Aus	0	..	..	..
		Aman	2	..	2	..
285	2	Aus	0	..	..	..
		Aman	2	..	2	..
286	8	Aus	0	..	..	..
		Aman	8	..	8	..
287	2	Aus	1	..	1	..
		Aman	1	1	..	..
288	1	Aus	0	..	..	..
		Aman	1	..	1	..
289	2	Aus	2	..	1	1
		Aman	0	..	..	..
290	2	Aus	2	..	2	..
		Aman	0	..	..	..
291	1	Aus	1	..	1	..
		Aman	0	..	..	..
292	2	Aus	1	..	1	..
		Aman	1	..	1	..
293	1	Aus	0	..	..	..
		Aman	1	..	1	..
294	1	Aus	1	..	..	1
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
295	7	Aus	7	1	6	..
		Aman	0	..	..	..
296	8	Aus	8	2	6	..
		Aman	0	..	..	..
297	4	Aus	3	2	1	..
		Aman	1	..	1	..
298	1	Aus	0	..	..	..
		Aman	1	..	1	..
299	1	Aus	1	..	1	..
		Aman	0	..	..	..
300	5	Aus	5	1	4	..
		Aman	0	..	..	..
301	10	Aus	10	4	6	..
		Aman	0	..	..	..
302	1	Aus	1	1	..	..
		Aman	0	..	..	..
303	2	Aus	0	..	..	..
		Aman	2	..	2	..
304	10	Aus	1	..	1	..
		Aman	9	..	9	..
305	2	Aus	2	..	1	1
		Aman	0	..	..	..
306	2	Aus	2	..	2	..
		Aman	0	..	..	..
307	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
308	2	Aus	0	..	..	..
		Aman	2	..	2	..
309	1	Aus	1	..	1	..
		Aman	0	..	..	..
310	2	Aus	2	..	2	..
		Aman	0	..	..	..
311	4	Aus	3	..	3	..
		Aman	1	..	1	..
312	1	Aus	0	..	..	..
		Aman	1	..	1	..
313	1	Aus	0	..	..	..
		Aman	1	..	1	..
314	1	Aus	0	..	..	..
		Aman	1	..	1	..
315	1	Aus	0	..	..	..
		Aman	1	..	1	..
316	1	Aus	0	..	..	..
		Aman	1	..	1	..
317	1	Aus	0	..	..	..
		Aman	1	..	1	..
318	1	Aus	1	..	1	..
		Aman	0	..	..	..
319	1	Aus	1	..	1	..
		Aman	0	..	..	..
320	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
321	1	Aus	1	..	1	..
		Aman	0	..	..	..
322	1	Aus	1	..	1	..
		Aman	0	..	..	..
323	1	Aus	1	..	1	..
		Aman	0	..	..	..
324	1	Aus	1	..	1	..
		Aman	0	..	..	..
325	1	Aus	1	..	1	..
		Aman	0	..	..	..
326	3	Aus	3	..	3	..
		Aman	0	..	..	..
327	2	Aus	1	..	1	..
		Aman	1	..	1	..
328	1	Aus	0	..	..	..
		Aman	1	..	1	..
329	1	Aus	0	..	..	..
		Aman	1	..	1	..
330	1	Aus	0	..	..	..
		Aman	1	..	1	..
331	2	Aus	2	..	2	..
		Aman	0	..	..	..
332	1	Aus	1	..	1	..
		Aman	0	..	..	..
333	1	Aus	0	..	..	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
334	1	Aus	1	..	1	..
		Aman	0	..	..	..
335	1	Aus	0	..	..	..
		Aman	1	..	1	..
336	3	Aus	3	3	..	..
		Aman	0	..	..	..
337	1	Aus	1	..	1	..
		Aman	0	..	..	..
338	1	Aus	0	..	..	..
		Aman	1	..	1	..
339	1	Aus	0	..	..	..
		Aman	1	..	1	..
340	1	Aus	0	..	..	..
		Aman	1	..	1	..
341	1	Aus	0	..	..	..
		Aman	1	..	1	..
342	1	Aus	0	..	..	..
		Aman	1	..	1	..
343	8	Aus	0	..	..	..
		Aman	8	..	8	..
344	3	Aus	0	..	..	..
		Aman	3	..	3	..
345	1	Aus	0	..	..	..
		Aman	1	..	1	..
346	1	Aus	0	..	..	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
347	1	Aus	0	..	..	..
		Aman	1	..	1	..
348	1	Aus	0	..	..	..
		Aman	1	..	1	..
349	1	Aus	0	..	..	..
		Aman	1	..	1	..
350	1	Aus	0	..	..	..
		Aman	1	..	1	..
351	1	Aus	0	..	..	..
		Aman	1	..	1	..
352	1	Aus	0	..	..	..
		Aman	1	..	1	..
353	1	Aus	1	1	..	..
		Aman	0	..	..	..
354	1	Aus	0	..	..	..
		Aman	1	..	1	..
355	1	Aus	1	..	..	1
		Aman	0	..	..	..
356	2	Aus	2	..	2	..
		Aman	0	..	..	..
357	1	Aus	0	..	..	..
		Aman	1	..	1	..
358	1	Aus	0	..	..	..
		Aman	1	..	1	..
359	2	Aus	2	..	2	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
360	3	Aus	3	..	2	1
		Aman	0	..	..	..
361	4	Aus	4	1	3	..
		Aman	0	..	..	..
362	1	Aus	1	..	1	..
		Aman	0	..	..	..
363	7	Aus	7	..	4	3
		Aman	0	..	..	..
364	2	Aus	2	..	2	..
		Aman	0	..	..	..
365	1	Aus	1	..	1	..
		Aman	0	..	..	..
366	2	Aus	0	..	..	..
		Aman	2	..	2	..
367	1	Aus	0	..	..	..
		Aman	1	..	1	..
368	1	Aus	0	..	..	..
		Aman	1	..	1	..
369	1	Aus	0	..	..	..
		Aman	1	..	1	..
370	1	Aus	1	..	1	..
		Aman	0	..	..	..
371	2	Aus	2	..	2	..
		Aman	0	..	..	..
372	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
373	2	Aus	2	..	2	..
		Aman	0	..	..	..
374	2	Aus	2	..	2	..
		Aman	0	..	..	..
375	1	Aus	0	..	..	..
		Aman	1	..	1	..
376	1	Aus	0	..	..	..
		Aman	1	..	1	..
377	3	Aus	0	..	..	..
		Aman	3	..	3	..
378	2	Aus	0	..	..	..
		Aman	2	..	2	..
379	1	Aus	0	..	..	..
		Aman	1	..	1	..
380	2	Aus	0	..	..	..
		Aman	2	..	2	..
381	2	Aus	0	..	..	..
		Aman	2	..	2	..
382	1	Aus	0	..	..	..
		Aman	1	..	1	..
383	2	Aus	2	..	2	..
		Aman	0	..	..	..
384	1	Aus	1	..	1	..
		Aman	0	..	..	..
385	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
386	1	Aus	0	..	..	..
		Aman	1	..	1	..
387	2	Aus	0	..	..	..
		Aman	2	..	1	1
388	1	Aus	1	..	1	..
		Aman	0	..	..	..
389	1	Aus	1	..	1	..
		Aman	0	..	..	..
390	5	Aus	5	3	2	..
		Aman	0	..	..	..
391	10	Aus	10	4	6	..
		Aman	0	..	..	..
392	1	Aus	0	..	..	..
		Aman	1	..	1	..
393	1	Aus	1	..	1	..
		Aman	0	..	..	..
394	1	Aus	1	1	..	..
		Aman	0	..	..	..
395	11	Aus	8	7	1	..
		Aman	3	..	3	..
396	23	Aus	18	6	12	..
		Aman	5	..	5	..
397	3	Aus	2	..	2	..
		Aman	1	..	1	..
398	1	Aus	1	1	..	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
399	2	Aus	2	..	2	..
		Aman	0	..	..	..
400	7	Aus	6	3	3	..
		Aman	1	..	1	..
401	14	Aus	12	5	4	3
		Aman	2	..	2	..
402	4	Aus	3	2	1	..
		Aman	1	..	1	..
403	1	Aus	1	1	..	..
		Aman	0	..	..	..
404	1	Aus	0	..	..	..
		Aman	1	..	1	..
405	1	Aus	1	..	..	1
		Aman	0	..	..	..
406	1	Aus	0	..	..	..
		Aman	1	..	1	..
407	2	Aus	1	..	1	..
		Aman	1	..	1	..
408	1	Aus	0	..	..	..
		Aman	1	..	1	..
409	4	Aus	3	..	3	..
		Aman	1	..	1	..
410	5	Aus	1	..	1	..
		Aman	4	..	4	..
411	2	Aus	0	..	..	..
		Aman	2	..	2	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
412	1	Aus	0	..	..	..
		Aman	1	..	1	..
413	1	Aus	0	..	..	..
		Aman	1	..	1	..
414	2	Aus	1	..	1	..
		Aman	1	..	1	..
415	1	Aus	0	..	..	..
		Aman	1	..	1	..
416	1	Aus	0	..	..	..
		Aman	1	..	1	..
417	3	Aus	2	..	2	..
		Aman	1	..	1	..
418	1	Aus	0	..	..	..
		Aman	1	..	1	..
419	1	Aus	1	..	1	..
		Aman	0	..	..	..
420	2	Aus	2	..	2	..
		Aman	0	..	..	..
421	1	Aus	0	..	..	..
		Aman	1	..	1	..
422	2	Aus	2	..	2	..
		Aman	0	..	..	..
423	2	Aus	2	..	2	..
		Aman	0	..	..	..
424	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
425	2	Aus	2	..	2	..
		Aman	0	..	..	..
426	2	Aus	2	..	2	..
		Aman	0	..	..	..
427	2	Aus	2	..	2	..
		Aman	0	..	..	..
428	1	Aus	1	..	1	..
		Aman	0	..	..	..
429	3	Aus	3	..	3	..
		Aman	0	..	..	..
430	1	Aus	1	..	1	..
		Aman	0	..	..	..
431	1	Aus	0	..	..	..
		Aman	1	..	1	..
432	1	Aus	0	..	..	..
		Aman	1	..	1	..
433	1	Aus	0	..	..	..
		Aman	1	..	1	..
434	1	Aus	0	..	..	..
		Aman	1	..	1	..
435	1	Aus	0	..	..	..
		Aman	1	..	1	..
436	1	Aus	1	..	1	..
		Aman	0	..	..	..
437	2	Aus	2	..	2	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
438	2	Aus	2	..	2	..
		Aman	0	..	..	..
439	1	Aus	1	..	1	..
		Aman	0	..	..	..
440	9	Aus	9	6	3	..
		Aman	0	..	..	..
441	8	Aus	8	6	2	..
		Aman	0	..	..	..
442	3	Aus	3	1	1	1
		Aman	0	..	..	..
443	2	Aus	2	2	..	..
		Aman	0	..	..	..
444	4	Aus	4	4	..	..
		Aman	0	..	..	..
445	3	Aus	3	1	2	..
		Aman	0	..	..	..
446	1	Aus	1	1	..	..
		Aman	0	..	..	..
447	1	Aus	1	..	1	..
		Aman	0	..	..	..
448	1	Aus	0	..	..	..
		Aman	1	..	1	..
449	2	Aus	0	..	..	..
		Aman	2	..	2	..
450	2	Aus	1	1	..	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
451	2	Aus	1	..	1	..
		Aman	1	..	1	..
452	9	Aus	1	..	1	..
		Aman	8	..	8	..
453	1	Aus	1	..	..	1
		Aman	0	..	..	..
454	2	Aus	2	..	1	1
		Aman	0	..	..	..
455	2	Aus	2	..	1	1
		Aman	0	..	..	..
456	1	Aus	1	..	1	..
		Aman	0	..	..	..
457	4	Aus	4	..	4	..
		Aman	0	..	..	..
458	11	Aus	11	1	10	..
		Aman	0	..	..	..
459	2	Aus	1	..	1	..
		Aman	1	..	1	..
460	1	Aus	1	..	1	..
		Aman	0	..	..	..
461	1	Aus	1	..	1	..
		Aman	0	..	..	..
462	1	Aus	0	..	..	..
		Aman	1	..	1	..
463	1	Aus	0	..	..	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
464	1	Aus	0	..	..	..
		Aman	1	..	1	..
465	1	Aus	1	..	1	..
		Aman	0	..	..	..
466	12	Aus	12	6	5	1
		Aman	0	..	..	..
467	10	Aus	9	2	6	1
		Aman	1	..	1	..
468	5	Aus	5	2	3	..
		Aman	0	..	..	..
469	1	Aus	1	1	..	..
		Aman	0	..	..	..
470	1	Aus	0	..	..	..
		Aman	1	..	1	..
471	2	Aus	2	2	..	..
		Aman	0	..	..	..
472	3	Aus	2	..	2	..
		Aman	1	..	1	..
473	2	Aus	0	..	..	..
		Aman	2	..	2	..
474	1	Aus	1	..	1	..
		Aman	0	..	..	..
475	1	Aus	1	..	..	1
		Aman	0	..	..	..
476	1	Aus	1	..	1	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
477	1	Aus	0	..	..	..
		Aman	1	..	1	..
478	1	Aus	0	..	..	..
		Aman	1	..	1	..
479	1	Aus	0	..	..	..
		Aman	1	..	1	..
480	1	Aus	1	..	1	..
		Aman	0	..	..	..
481	2	Aus	2	..	2	..
		Aman	0	..	..	..
482	4	Aus	4	..	4	..
		Aman	0	..	..	..
483	1	Aus	1	..	1	..
		Aman	0	..	..	..
484	1	Aus	1	..	1	..
		Aman	0	..	..	..
485	2	Aus	2	..	2	..
		Aman	0	..	..	..
486	1	Aus	1	..	1	..
		Aman	0	..	..	..
487	1	Aus	0	..	..	..
		Aman	1	..	1	..
488	1	Aus	1	..	1	..
		Aman	0	..	..	..
489	2	Aus	0	..	..	..
		Aman	2	..	2	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
490	1	Aus	1	..	1	..
		Aman	0	..	..	..
491	2	Aus	2	..	2	..
		Aman	0	..	..	..
492	1	Aus	0	..	..	..
		Aman	1	..	1	..
493	1	Aus	1	..	1	..
		Aman	0	..	..	..
494	2	Aus	2	2	..	..
		Aman	0	..	..	..
495	3	Aus	3	2	1	..
		Aman	0	..	..	..
496	1	Aus	1	1	..	..
		Aman	0	..	..	..
497	2	Aus	2	1	1	..
		Aman	0	..	..	..
498	1	Aus	1	..	1	..
		Aman	0	..	..	..
499	1	Aus	1	..	1	..
		Aman	0	..	..	..
500	1	Aus	1	..	1	..
		Aman	0	..	..	..
501	3	Aus	3	..	3	..
		Aman	0	..	..	..
502	9	Aus	8	1	7	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
503	2	Aus	2	..	2	..
		Aman	0	..	..	..
504	2	Aus	2	..	2	..
		Aman	0	..	..	..
505	3	Aus	3	..	3	..
		Aman	0	..	..	..
506	3	Aus	2	..	2	..
		Aman	1	..	..	1
507	3	Aus	3	1	2	..
		Aman	0	..	..	..
508	1	Aus	0	..	..	..
		Aman	1	1	..	..
509	1	Aus	1	..	1	..
		Aman	0	..	..	..
510	1	Aus	0	..	..	..
		Aman	1	1	..	..
511	1	Aus	0	..	..	..
		Aman	1	..	1	..
512	1	Aus	0	..	..	..
		Aman	1	..	1	..
513	1	Aus	1	..	1	..
		Aman	0	..	..	..
514	2	Aus	2	..	2	..
		Aman	0	..	..	..
515	1	Aus	0	..	..	..
		Aman	1	..	1	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
516	1	Aus	0	..	..	..
		Aman	1	..	1	..
517	1	Aus	0	..	..	..
		Aman	1	..	1	..
518	1	Aus	0	..	..	..
		Aman	1	..	1	..
519	1	Aus	0	..	..	..
		Aman	1	..	1	..
520	1	Aus	0	..	..	..
		Aman	1	..	1	..
521	1	Aus	0	..	..	..
		Aman	1	1	..	..
522	1	Aus	0	..	..	..
		Aman	1	..	1	..
523	2	Aus	2	2	..	..
		Aman	0	..	..	..
524	1	Aus	1	1	..	..
		Aman	0	..	..	..
525	1	Aus	1	..	1	..
		Aman	0	..	..	..
526	2	Aus	0	..	..	..
		Aman	2	..	2	..
527	1	Aus	1	1	..	..
		Aman	0	..	..	..
528	1	Aus	1	1	..	..
		Aman	0	..	..	..

Appendix II—*contd.*

Variety No.	No. of types	*Aus* and *Aman*		Early	Medium	Late
529	1	Aus	1	..	1	..
		Aman	0	..	..	..
530	1	Aus	1	..	1	..
		Aman	0	..	..	..
531	1	Aus	1	..	1	..
		Aman	0	..	..	..
532	2	Aus	2	1	1	..
		Aman	0	..	..	..
533	2	Aus	2	1	1	..
		Aman	0	..	..	..
534	1	Aus	1	..	1	..
		Aman	0	..	..	..
535	1	Aus	1	..	1	..
		Aman	0	..	..	..
536	1	Aus	1	..	1	..
		Aman	0	..	..	..
537	1	Aus	0	..	..	..
		Aman	1	..	1	..
538	1	Aus	1	1	..	..
		Aman	0	..	..	..
539	1	Aus	1	..	1	..
		Aman	0	..	..	..
540	1	Aus	0	..	..	..
		Aman	1	..	1	..

INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET.

IV. BROWN GRAINS.

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(With Plate VIII)

In a previous article [Rangaswami Ayyangar *et al*, 1933] the relationship between the Red, Yellow and White grains has been given. In this article the factors determining the production of brown grains and the relationship of the brown grain to other grains will be examined utilizing the data collected from 1922 onwards.

Monogenic segregations have been met with between the following pairs of grain colours :—

Brown .. .. .. White

Brown .. .. .. Yellow

Brown .. .. .. Red

The following table presents the families and the numbers obtained for the above segregations :—

TABLE I.

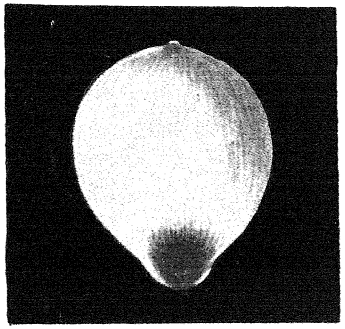
Family numbers	Grain colours	
A	Brown	White
A. S. 901, 1516, 1885, 1989, 2190, 2297, 2382, 2384, 2392, 2703, 2705, 2709, 2710, 2720, 2899, 2900.	1300	444
Expected (3 : 1)	1308	436
$\chi^2 = 0{\cdot}196$; $P > 0{\cdot}5$		
B	Brown	Yellow
A. S. 186, 427, 1287, 1288, 1364, 1791, 1878, 1879, 2073, 2179, 2271, 2276, 2279, 2310, 2311, 2312, 2654.	1039	343
Expected (3 : 1)	1036·5	345·5
$\chi^2 = 0{\cdot}024$; $P > 0{\cdot}8$		
C	Brown	Red
A. S. 2616, 2617, 2659, 2831, 2832, 2835, 2836, 2878 . . .	769	265
Expected (3 : 1)	775·5	258·5
$\chi^2 = 0{\cdot}218$; $P > 0{\cdot}5$		

Digenic segregations connoting the existence of two factors for the production of brown, have been obtained, for the same pairs of grain colours.

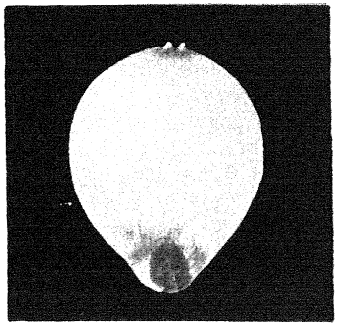
TABLE II.

Family numbers		Grain colours	
	A	Brown	White
From natural crosses	*A. S.* 1324, 1348, 1349, 1350, 1400, 1920, 1921, 2001, 2371, 2398, 2399, 2400, 2548, 2715, 2718, 2719.	941	739
From artificial crosses	*A. S.* 2955, 2957, 2959, 2962, 2963 .	475	340
	Total .	1416	1079
	Expected (9 : 7) . . .	1403·4	1091·6
	$\chi^2 = 0{\cdot}259$; $P > 0{\cdot}5$		
	B	Brown	Yellow
From natural crosses	*A. S.* 1280, 1358, 1362, 2354 . .	211	152
From artificial crosses	*A. S.* 2929 to 2948	2995	2411
	Total .	3206	2563
	Expected (9 : 7) . .	3245·1	2523·9
	$\chi^2 = 1{\cdot}077$; $P = 0{\cdot}3$ (nearly)		
	C	Brown	Red
From natural crosses	*A. S.* 1997, 2657, 2660, 2840 . .	283	201
	Expected (9 : 7) . . .	272·25	211·75
	$\chi^2 = 0{\cdot}971$; $P > 0{\cdot}3$		

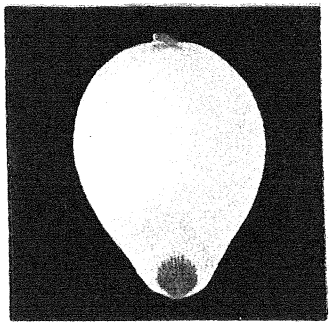
SORGHUM: BROWN FACTORS—PERICARP AND DRY ANTHER.



Brown washed Yellow



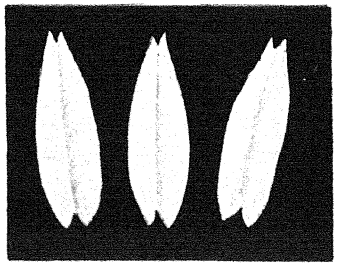
Good Yellow



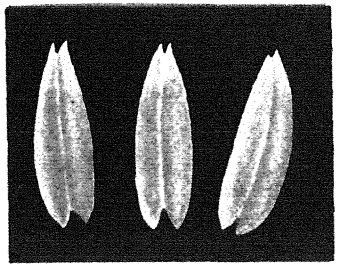
White



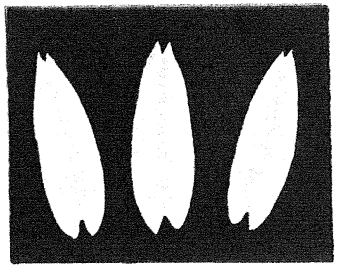
Brown



Sienna



Light Brown



Light Sienna

From the above tables it will be obvious that two factors are responsible for the production of the brown on the grain so that the full brown is not obtained without their concurrent presence. These factors have been designated $\mathbf{B_1}$ and $\mathbf{B_2}$.

The first set of monogenic segregations indicate a single factor difference. In the brown-white segregations, both the factors for brown are present in the brown grain, and in the white grains one or neither of the brown factors occur. The manifestation of even the one brown factor when present is not possible in the absence of the **W** factor determining the expression of pericarp colour in wholeness.

In the monogenic brown-yellow segregations the situation is simple in that both have pericarp colour. The browns have both the brown factors and are therefore of the usual good brown colour. In the yellows one or neither of the brown factors occur. The examination of a number of yellows has given two groups, namely good yellows, and yellows with a brown wash. Their separation is possible and rendered certain with the help of dry anthers which in the case of yellow with brown wash, take a brownish tint, as opposed to the (yellowish) sienna of the pure yellows (Plate VIII). The following segregations of yellows, with and without brown wash, have occurred in conjunction with segregations for dry anther colour.

TABLE III.

Pure for **Y** *and* **W**, *and segregating for one* **B** *factor.*

Family numbers	Grain colour	Brown washed yellow	Good yellow
	Dry anther	Brown	Sienna
A. S. 1187, 1197, 1198, 1223, 1243, 1478, 2078, 2079, 2582, 2584, 2587, 2589, 2590 .	..	846	293
Expected (3 : 1) .	..	854·25	284·75

$\chi^2 = 0{\cdot}319$; $P > 0{\cdot}5$

In the next table is presented a family *A. S.* 1838, segregating for yellow and white grain colour with and without a **B** factor.

TABLE IV.

Family No. A. S. 1838.

(Natural cross spotted in 1926).

Pure for **Y** *and segregating for* **W** *and one* **B** *factor.*

Generation	Family Nos.	Character of selection	Behaviour of progeny			
		Grain	Brown washed yellow	Good yellow	White	
		Dry anther	Brown	Sienna	Light brown	Light sienna
F_2	*A. S.* 1838	Brown washed yellow, brown.	59	16	26	
F_3	,, 2250	,,	59	20	20	
	,, 2247	,,	94	27	..	..
	,, 2249	,,	74	17	..	..
	,, 2248	,,	pure	..	..	..
	,, 2251	Good yellow, sienna	..	44	..	13
	,, 2252	,,	..	33	..	16
	,, 2253	,,	..	35	..	7
	,, 2254	White, light brown	..	..	32	18
F_4	(from *A. S.* 2250)					
	A. S. 2790	Brown washed yellow, brown.	98	38	30	13
	,, 2793	,,	76	47	24	8
	,, 2794	,,	79	30	23	5
	,, 2795	,,	86	27	25	13
	,, 2792	,,	119	..	30	..
	,, 2791	,,	pure	..	..	..
	(from *A. S.* 2254)					
	A. S. 2628	White, light brown	..	..	49	19
	,, 2629	,,	..	..	39	8
	,, 2630	,,	..	..	47	17
	,, 2627	,,	..	..	pure	..
	,, 2631	White, light sienna	..	..	..	pure
	,, 2632					

In the above table the segregation of the white grain into dry anther colours with and without brown is very interesting and clearly points to the existence of factors for brown in the white-grained plant, not expressible on the pericarp (through lack of **W** factor), but demonstrable through the more subtle dry anther colour (Plate VIII).

In the monogenic brown-red segregations the overpowering red colour does not permit of a sharp separation between the shades of reddish-brown brought about by a single **B** factor difference.

The above explanations of the manifestation of single brown factors, help us to understand the digenic difference between brown and red presented in Table II, above. In the artificial crosses given in that Table, two whites have been crossed and produced a brown grain, similarly two yellows have been crossed and gave a brown grain, in the first generation. Obviously the two brown factors $\mathbf{B}_1$ and $\mathbf{B}_2$ have been brought together. In the 9 : 7 ratio of brown to red, the two factors $\mathbf{B}_1$ and $\mathbf{B}_2$ have been brought together through red grains.

In the yellows the wash is clear and the component parts of the seven, 3, 3 and 1, in the shape of grains with and without the brown wash were obtained in six families in the expected ratio of six yellow grains with brown wash to one yellow without brown wash, the actual figures being 183 and 27 respectively. ($\chi^2=0{\cdot}35$; $P > 0{\cdot}5$).

In the following table the **B** factors have come together through red and white grains and explain the ratio of 27 : 9 : 21 : 7 met with.

TABLE V.

Artificial cross No. A. S. LVIII.

	Grain colours			
	Reddish brown	Brown	Red	White
Parents *A. S.* 590	..	..	..	♀
„ 1397	..	♂	..	..
F_1 „ LVIII	Reddish brown	..	..	..
F_2	149	58	121	33
Expected (27 : 9 : 21 : 7) .	152·3	50·8	118·4	39·5
$\chi^2=2{\cdot}219$; $P > 0{\cdot}5$				

From the above data it will be evident that the good brown colour in the grain is the resultant of the concurrent presence of two factors, B_1 and B_2. When the factor **W** is present and the whole grain is coloured, each of the **B** factors gives a brown wash to the grain over the basic colour. In plants producing white grains the presence of the **B** factor is detected only through the brown anther, and in yellow grains by a brown wash over the yellow. Red smothers it. Each of these colours may carry or not either or none of the **B** factors. When the two brown factors are brought together through two white grains lacking the **W** factor, the resultant F_1 is the ordinary brown. When they come together as washes on yellows, the resultant grain is a good brown. When the **B** factors come together dominated by red, the F_1 is a reddish brown.

In the following tables the inter-relationship in the play of the B_1 and B_2 factors over a red, yellow, and white background are presented :—

TABLE VI.

Pure for **W** *and* **Y** *and segregating for* **R**, B_1 *and* B_2.

Family numbers	Grain colours		
	Brown	Red	Yellow
A. S. 1761, 2543	54	38	13
Expected (36 : 21 : 7)	59	34·5	11·5
$\chi^2 = 0{\cdot}074$; $P > 0{\cdot}5$			

In the naked grain of sorghum in the absence of very favourable weather conditions, it is difficult to distinguish between brown and reddish-brown, but with the help of anthers the separation could be fixed up far ahead of the disturbing effects of the weather on the mature grain. Such separability will be evident from the following table :—

TABLE VII.

Family numbers	Grain and anther colours	
	Reddish brown	Brown
A. S. 186, 1295	83	26
Expected (3 : 1)	81·75	27·25
$\chi^2 = 0{\cdot}076$; $P > 0{\cdot}7$		

The following table illustrates the effect of the **B** factors on yellow and white undisturbed by the **R** factor :—

TABLE VIII.

Segregating for **W** *and one* **B** *factor.*

Family numbers	Grain colours		
	Brown	Yellow	White
A. S. 1785, 1792, 1927, 2205, 2224 . . .	302	88	25
Expected (12 : 3 : 1)	311·3	77·8	25·9

χ^2 = 1·647; P > 0·3

While it was found difficult to separate the reddish brown from brown for the reason that they were both pure for **W** and had the overpowering red, the separation of the browns of the above table was a bit easier in the absence of red and the play of **W**, aided by the corresponding clearer anther differences.

TABLE IX.

Pure for **B** *factors and segregating for* **W**.

Family numbers	Grain—dry anther	Good brown / Brown	Brown / Light brown
A. S. 1784, 1927, 2025, 2203, 2204, 2205, 2222, 2223, 2314	..	512	170
Expected (3 : 1)	..	511·5	170·5

χ^2 = 0·002; P > 0·9

Segregations have been met with for all the four grain colours—brown, red, yellow and white—in some families falling into the two groups, namely, those involving both the **B** factors, and those with a single **B** factor (being pure for the other) indicative of the expected ratios of 144 : 63 : 21 : 28 in the former, and 48 : 9 : 3 : 4 in the latter.

DISCUSSION.

The above data and their explanation necessitate a discussion of previous results and a reconciliation of these with the present one. Vinall and Cron [1921]

found a bi-factorial expression of brown. They refer to an under-coat of brown in some white grains. Their 15 : 1 ratio of coloured to white grains is obviously an interplay of one **B** factor and **W**, though no such explanation is given. Sieglinger [1924] explains his brown grain experiences in terms of the existence of a brown nucellar layer above the aleurone layer, possibly similar to the brown under-coat of Vinall and Cron. This basic presence is made manifest into pericarp brown, and further ramifications with other colours are thus made possible. Swanson [1928], after microscopic examination, elaborated Sieglinger's experiences by letting in (1) the presence or absence of the nucellar layer and its brown, (2) a thick or thin mesocarp masking the expression of the above colour, and (3) a factor determining epidermal and hypodermal pigmentation.

In our experience the nucellar brown has not been met with, and as such what variations there are in the mesocarp did not influence the situation. The absence of references to brown washes of a light coloured type in American literature lends belief to such an expression of an initial brown in the varieties we have examined, to be the parallels of the nucellar brown of the American experience. The absence of all easily detectable clues to the existence of the hidden brown in the pericarp of the whites, shows how expressionless a single dose of brown has become in the absence of the **W** factor determining pericarp expression of colour. It is only the delicate anthers that give the index to their presence.

A chemical examination of the brown grains shows that the pigment is not anthocyanic. Many of the vernacular names of brown grain varieties connote the astringency associated with brown grains.. It is possible that super-imposed on the anthocyanic expressions red and yellow, these browns, possibly tannic in affinities, have an independent play producing brownish blends of red and yellow grains. Many of the yellow-grained agricultural varieties have on the grain a brown wash indicating the presence of a single **B** factor. The persistence of this **B** factor in its light manifestation can be interpreted as an aid to the pure yellow-grained plant helping it to survive as an economic variety. Free crossing between brown and yellow races and subsequent selection are indicated as possibilities in the evolution of these economic varieties. Brown is the dominant colour in wild sorghums. The origin and distribution of sorghum varieties now under survey for the Millets Breeding Station may throw light on this interesting occurrence.

Summary.

Two factors B_1 and B_2 are responsible for the production of a full brown colour on the grain, produced by their concurrent presence.

Each of the **B** factors is capable of giving a light brown wash to the grain provided the **W** factor determining the expression of pericarp colour is also present.

In white grained varieties the **B** factors can be detected only through the colour of the dry anther, so much so that chance matings bring about brown grains in crosses between some white-grained varieties. This possibly explains the spontaneous occurrence of browns gradually vitiating a pure white-grained crop.

The interplay of the **B** factors which are not anthocyanic, over and above the anthocyanic red and yellow and their colourless allelomorphs, leads to the wealth of blended colours met with in sorghum.

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INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET.

V. LINKAGE BETWEEN SHEATH—GLUME AND DRY ANTHER—GRAIN COLOURS

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(With Plate IX)

In a previous paper [Rangaswami Ayyangar *et al*, 1933] the existence of relationship between leaf-sheath and glume colours has been stated. A reddish-purple sheath goes with a redish-purple glume. A blackish-purple sheath goes with a blackish-purple glume. The former (factor **Q**) is dominant to the latter (**q**).

In another article [Rangaswami Ayyangar *et al*, 1934], two factors $\mathbf{B}_1$ and $\mathbf{B}_2$ for the production of brown colour in the pericarp of the grain have been noted, each giving a mere brown wash to the grain. In the absence of the **W** factor, determining expression of colour in the pericarp, these brown factors show themselves through the brown tint that they impart to the otherwise sienna-coloured dry anther. **B** is dominant to **b**.

A remarkable relationship exists between the leaf-sheath colour factors and the brown colour factors referred to above. A close linkage (repulsion), probably absolute, between the genes for leaf-sheath colour (**Qq**) and the genes for brown in the anther (**Bb**) has been found to occur.

In the crosses presented in the following tables parents of the genetic constitution **QQbb** and **qqBB** figure. It will be noticed that the dominant factors are coming together not from the same parent, but one from each.

Since the effect of the operation of factors $\mathbf{B}_1$ and $\mathbf{B}_2$ is similar in the production of brown in the anther, and on the pericarp (when **W** is present), it is difficult to say which one of these is involved. Further crosses have been designed to help in this determination.

In Table I is presented the result of the operation of this linkage in white grains, the subtle anthers providing the clue. The lightness of the colour in the anthers is here due to the absence of the **W** factor.

TABLE I.

White grains.

Yw (Qb) × Yw (qB).

(From natural crosses spotted in 1925.)

Family No.	Character of selection	Behaviour of progeny			
	Sheath and glume	Reddish purple		Blackish purple	
	Dry anther	Light brown	Light sienna	Light brown	Light sienna
F_2—*A. S.* 1142 .	Reddish purple. Light brown.	62	34	33	..
F_3—*A. S.* 1586 .	,, ,, .	46	25	19	..
,, 1587 . .	,, ,, .	44	19	21	..
,, 1588 . .	,, ,, .	38	11	13	..
,, 1591 . .	,, ,, .	26	16	16	..
,, 1592 . .	Reddish purple. Light sienna.	..	pure	..	..
,, 1589, 1590, 1594, 1595	Blackish purple. Light brown.	..	..	pure	..
F_4—(from *A. S.* 1587)					
A. S. 2117 . .	Reddish purple. Light brown.	51	20	19	..
,, 2118 . .	,, ,, .	69	20	20	..
,, 2119 . .	,, ,, .	63	32	35	..
,, 2120 . .	,, ,, .	63	35	31	..
,, 2121, 2122	Reddish purple. Light sienna.	..	pure	..	..
,, 2123, 2124	Blackish purple. Light brown.	..	..	pure	..
	Total .	462	212	207	..
	Expected (2 : 1 : 1 : 0)	440·5	220·25	220·25	..

$\chi^2 = 2{\cdot}155$, $P > 0{\cdot}3$

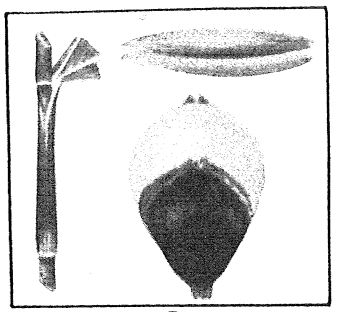
In Table II the same phenomenon is noticeable in yellow grains, there being, owing to the presence of the **W** factor, both brown in the anther and a brown wash on the grain (Plate IX).

TABLE II.

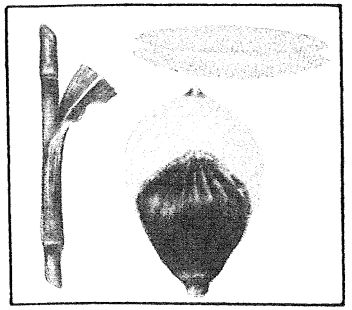
Yellow grains.—**YW (Qb)** × **YW (qB).**

(From natural cross spotted in 1925.)

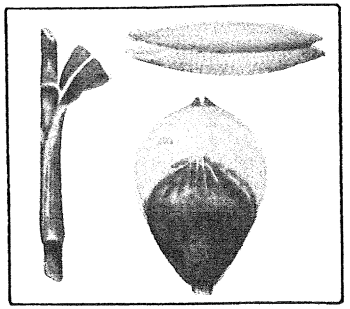
Family Nos.	Character of selection	Behaviour of progeny			
	Sheath and glume	Reddish purple		Blackish purple	
	Grain	Yellow Br. wash	Yellow	Yellow Br. wash	Yellow
	Dry anther	Brown	Sienna	Brown	Sienna
F_2—*A. S.* 1244.	Reddish purple, yellow br. wash, brown.	84	54	52	..
F_3— „ 1839	„ „	47	20	26	..
„ 1840	„ „	55	36	27	..
„ 1841	„ „	43	26	19	..
„ 1842	Reddish purple, yellow, sienna.	..	pure	..	..
„ 1843	„ „				
„ 1844	Blackish purple, yellow brown wash, brown.	..	..	pure	..
„ 1845	„ „				
F_4 (from *A. S.* 1839) *A. S.* 2255	Reddish purple, yellow brown wash, brown.	126	63	63	..
„ 2256	„ „	103	34	39	..
„ 2257	„ „	128	57	44	..
„ 2258	„ „	119	56	44	..
		705	346	314	..
Other families :— *A. S.* 1253, 1274, 1665, 1862, 1863, 1866, 1873, 1874, 1875, 2145, 2151, 2152, 2153, 2154.		942	456	469	..
Total		1,647	802	783	..
Expected (2 : 1 : 1 : 0)		1,616	808	808	..
		$\chi^2 = 1{\cdot}414$; $P = 0{\cdot}49$			



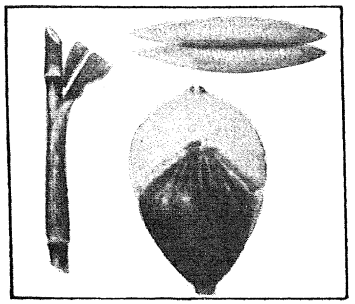
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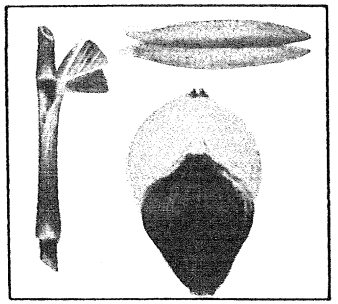
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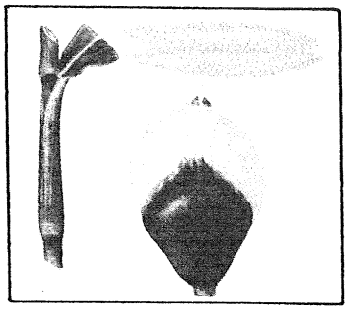
F_1



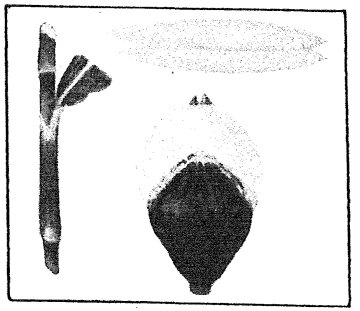
F_2 2



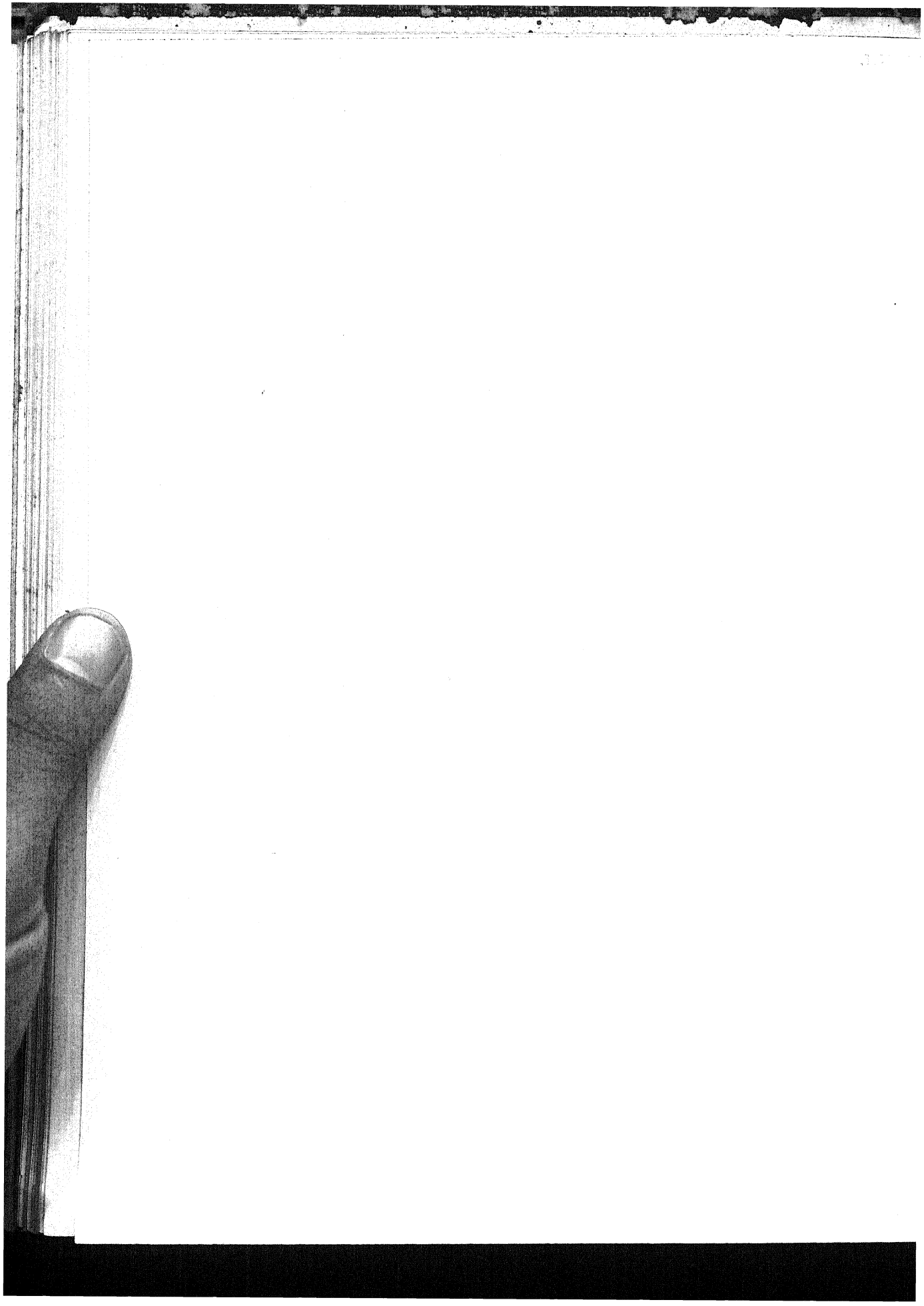
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Artificial crosses have been made with suitable parents, and the behaviour of one of these crosses presented below supports the above experiences :—

TABLE III.

Yellow grains. **YW(Qb)×YW(qB).**

(Artificial cross No. A. S. I.—1927.)

Family Nos.	Character of selection	Behaviour of progeny			
	Sheath and glu me	Reddish purple		Blackish purple	
	Grain	Yellow Br. Wash	Yellow	Yellow Br. Wash	Yellow
	Dry anther	Brown	Sienna	Brown	Sienna
Parents— *A. S.* 1842 .	..	..	♀	..	..
,, 1844 .	..	..	..	♂	..
F_1 ,, I .	..	Reddish purple, yellow br. wash, brown.	..	..	..
F_2 ,, 2916 .	Reddish purple, yellow br. wash, brown.	150	67	61	..
,, 2917 .	Ditto .	54	26	26	..
,, 2918 .	Ditto .	68	43	36	..
,, 2919 .	Ditto .	75	26	44	..
	Total .	347	162	167	..
	Expected (2 : 1 : 1 : 0).	338	169	169	..
		$\chi^2=0{\cdot}553$; $P > 0{\cdot}7$			..

In Table IV the same linkage is noticed in the presence of the **R** factor producing pink grains. The red of the anthers is not strong enough to smother the expression of brown. It is needless to add that with the addition of the **I** factor to pink the resultant overpowering red will make the pursuit of the brown an impossible task.

TABLE IV.

Pink grains. YRW(Qb)×YRW(qB).

(From natural cross spotted in 1927.)

Family Nos.	Character of selection	Behaviour of progeny			
	Sheath and glume	Reddish purple		Blackish purple	
	Grain	Pink Br. Wash	Pink	Pink Br. Wash	PINK
	Dry anther	Red. Brown	Red	Red. Brown	Red
F_2—*A. S.* 2141 .	Reddish purple, pink br. wash, red. brown.	68	36	38	..
„ 2142 . .	Ditto .	91	42	47	..
„ 2143 . .	Ditto .	62	29	27	..
„ 2144 . .	Ditto .	63	33	35	..
F_3 (from *A. S.* 2142)—					
„ „ 2520 .	Ditto .	318	141	148	..
„ „ 2521 .	Ditto .	338	149	176	..
„ „ 2522 .	Ditto .	248	150	152	..
„ „ 2523 .	Ditto .	262	133	122	..
„ „ 2524 .	Ditto .	248	129	136	..
„ „ 2525 .	Ditto .	250	106	107	..
„ „ 2526 .	Ditto .	340	127	124	..
„ „ 2527 .	Ditto .	291	139	161	..
„ „ 2528 .	Blackish purple, pink br. wash, red. brown.	..	..	Pure	..
„ „ 2529 .	Reddish purple, pink. red.	..	Pure	..	..
	Total .	2579	1214	1273	..
	Expected (2 : 1 : 1 : 0)	2533	1266·5	1266·5	..
		$\chi^2=3{\cdot}045$; $P>0{\cdot}2$			..

All the above tables presenting a 2 : 1 : 1 : 0 distribution instead of the usual digenic 9 : 3 : 3 : 1, mark this phenomenon as one of complete linkage. The group of double dominants with reddish-purple leaf-sheath and brown in the anther, have all proved always heterozygous, the other two parental combinations proving always homozygous. It will be noticed that in the population of about 10,000 of the above tables, there occurred no instance of a cross-over with blackish-purple sheaths, and anthers or grains, without brown.

This interesting fact is the first record of linkage in the grain and plant characters of sorghum, imposing limitations on the freedom of mating and fixing new combinations of characters.

Other aspects of this interesting linkage are under examination at the Millets Breeding Station.

Summary.

In sorghum, there is a linkage, probably complete, between **Qq** (factors for leaf-sheath and glume colour) and **Bb** (factors for brown colour in dry anther and grain).

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Rangaswami Ayyangar. G. N.; Vijiaraghavan, C.; Sankara Ayyar, M. A.; Panduranga Rao, V. and Subramanyam, P. (1934). *Ibid* 4, 81-89.

INHERITANCE OF CHARACTERS IN SORGHUM—THE GREAT MILLET.

VI. PEARLY AND CHALKY GRAINS.

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(Received for publication on 17th May 1933)
(With Plate X)

In the previous articles factors determining the colour of the sorghum grain have been described. In this article an aspect of the external appearance, apart from colour, will be examined.

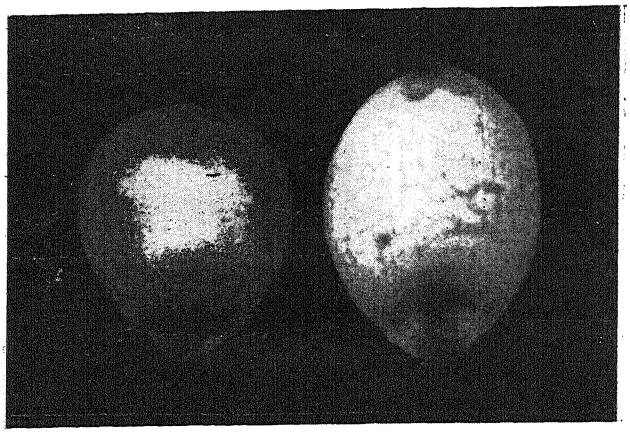
The common sorghum grain devoid of all colour is 'pearly' in appearance, in most cases shining and translucent. There is considerable variations in this pearliness, but the group as such is sharp in contrast with its sister character 'chalky'.

The chalky grain lacks this lustre, looks like chalk and is opaque. The intensity of this chalkiness differs between varieties and the setting which the glumes give to such chalkiness in the grain helps often to show off this opacity. These chalky grains are not so common in the popular sorghums but they do exist as varieties in current cultivation. Such vernacular names as *uppu vellai*—salt white, connote this opacity in contrast to the name, *ennai vellai*—oily white, connotative of the pearly lustre of the other type.

Whereas the Durras have a few chalky grains, these chalkies are met with in abundance in the group *Roxburghii* var. *Hians*. The longer duration of many of the sorghums of the Hians group possibly allows of a greater time for a slow deposition of starch. In recent importations *Sorghum margaretiferum* from Sierra Leone has some chalky types. Many of these are borne in glumes that gape out on maturity and almost all of these have brown in the ripening colour of their anthers, so much so, that the chalky character and its incursion into the Durra grain sorghums could possibly be traceable to the Hians group.

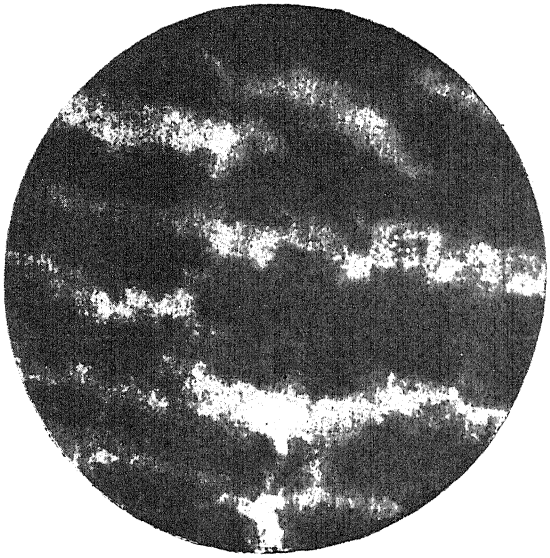
PLATE X.

SORGHUM: PEARLY AND CHALKY GRAIN.



Pearly. Chalky.

Fig. 1.



Chalky—magnified.

Fig. 2.

These chalky grains are structurally different from the pearly grains in having a mesocarp about thrice as thick as that in pearly grains. This mesocarp is full of starch grains and consequently the water absorption of the chalky grains is greater and quicker than is the case with pearly grains [Swanson, 1926]. In the soaking experiments carried out at the Millets Breeding Station in the grain sorghums, chalky grains absorbed 24 per cent. and pearly grains 18 per cent. of water over their dry weight, in a period of 5 hours. The chalky grains germinated a bit quicker. It would thus be seen that the chalky grains are more fitted for light soils with low moisture retaining qualities.

It was noticed that these chalky grains were relatively more susceptible to weevil attack than the pearly grains. For popping, one of the uses to which the sorghum grain can be put, it has been found that the pearly grains pop better and freer than chalky ones.

The characteristic feature of the chalky grains is a banded distribution of this chalkiness on the grain. To a casual look it presents a corrugated appearance, with furrows of a lighter colour (Plate X). But a microscopic examination of the grain reveals that the seed-coat is smooth and that the irregularity is in the deposition of starch, areas of starch deposit alternating with empty disintegrated cells, devoid of starch. This alternation gives the corrugated look. Mesocarp structures cleared of starch with potassium hydroxide eliminated this corrugation. This banded appearance is a great help in the location of the starchy character in coloured grains in which unlike the whites the cumulative effect of this deposition of starch, does not help to mark off the pearly from the chalky grains.

This starchy condition of the thick mesocarp showing itself chalky in appearance has proved a simple recessive to the thinner non-starchy mesocarp giving a pearly appearance. These pearly and chalky characters have been designated **Z** and z respectively. For a number of years simple monogenic segregations have been obtained between pearly and chalky grains in white grain varieties...

TABLE I.

Segregating for **Z**.

Family numbers	White grain	
	Pearly	Chalky
A. S.—1146, 1147, 1304, 1391, 1393, 1394, 1795, 1796, 1935, 1986, 1987, 2231, 2232, 2233, 2234, 2236, 2383, 2784, 2785, 2786, 2787, 2849, 3212, 3214, 3215, 3216, 3217, 3218	1889	628

In a family segregating for yellow and white grains—*A. S.* 1332, a 12 : 3 : 1 ratio of yellow : white pearly : white chalky was obtained, the figures being 62, 15 and 5.

Similar 12 : 3 : 1 ratios have been met with in a number of families segregating for red, white pearly and white chalky grains.

TABLE II.

Pure for **R** *and segregating for* **W** *and* **Z**.

Family numbers	Grain		
	Red	White pearly	White chalky
A. S.—1331, 2245, 2778, 2779. 2780, 2782	573	146	47

With increased knowledge about the nature of chalkiness the sub-division of the reds into their component groups was attempted with the following result :—

TABLE III.

Pure for **R** *and segregating for* **W** *and* **Z**.

Family numbers	Grain			
	Red		White	
	Smooth	Banded	Pearly	Chalky
A. S.—3204, 3207, 3210	142	39	47	16

It will thus be seen that even coloured grains could be classified into pearly and chalky groups according to the evenness or corrugated appearance which the uneven starch deposits in a thick mesocarp give them. This separation is rather difficult owing to the troubles incidental to weathering, but in many families segregating for red, yellow and white grains, the presence of both pearly and chalky grains in the

white group has always given the clue to the existence of pearly and chalky sub-groups among the coloured grains.

In coloured grains, brown is the heaviest and has the least transparency about it. Even so the banded condition of the chalkies among brown grains can be spotted with a lens, there being deposits of marked brown in the "furrows". But a clear separation of the pearlies and chalkies in brown grains is full of risks through want of clarity in pursuit. The best test for separating browns into pearly and chalky is the isolation and fixing of brown types whose allelomorphs have been white grains either pearly or chalky.

As has already been stated the chalky condition is largely traceable to the Hians group as also most manifestations of the brown factors. There is thus a peculiar intimacy in the association of the pearly-chalky characters and factors for brown in the grain. These are under study and the results will be reported in further papers.

Summary.

Sorghum grains fall into the two broad groups 'Pearly' and 'Chalky'. The former is translucent and the latter opaque. Chalky grains are characterised by a large deposit of starch in their thick mesocarp. The deposit being uneven, gives the grain a banded appearance. The opaque grains seem, by virtue of their quicker absorption of water, to be specially suited for sowing in light loams. Pearly (**Z**) character is a simple dominant to the chalky (**z**) character. Coloured grains also manifest this banded appearance when chalky. A separation can be attempted in lighter colours, but with the depth in colouring the colourless allelomorphs have to be depended upon for this classification of coloured grains into pearly and chalky.

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Swanson, A. F. (1926). *J. Amer. Soc. Agron.* **18**, 428.

COTTON ANTHRACNOSE IN THE CENTRAL PROVINCES.

BY

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(Received for publication on 11th September 1933)

(With Plates XI and XII and three text-figures)

Cotton anthracnose has been known in many cotton growing countries, but it is not certain if the fungus that causes this disease in India, especially in the Central Provinces and in the Madras Presidency, is the same as the one causing anthracnose in the United States, Egypt, Burma and other countries. Butler [1918] reports that the anthracnose disease, caused by *Glomerella gossypii* (Southw.) Edg., is " rare in India though it is exceedingly common and does some damage in the United States ". In Madras and the Central Provinces there is another anthracnose disease, caused by *Colletotricum* sp. Sundararaman [1927] has reported this disease from Madras since 1926-27. In these Provinces it has been known for a long time though the damage done has been usually insignificant ; but in 1931 it was quite wide-spread in an epidemic form in the different cotton growing districts, and it caused considerable damage to bolls, lint and seed. The bolls that were first to set in September and October were the most affected ; those that were formed later were less infected, and the infection decreased, and ultimately completely disappeared, with the end of the rains and the beginning of the cold weather. In 1931 the rainfall was unusually high ; the rainy season extended upto the first week of November ; there was heavy rainfall from August to the first week of November, at times for many days together. Even when there was no precipitation, the days were often cloudy and therefore sunny days were few and far between with the result that the atmosphere for long periods during the months of September, October and November was charged with high humidity.

Table I gives the total rainfall from June 1st to November 30th for the years 1928 to 1932 and the number of days there was precipitation in the months of August to November at Nagpur and Akola. Table II gives the rainfall and deviation from normal in the months of August to November of 1928 to 1932 at Nagpur, Akola and Khandwa.

TABLE I.

Year	Total rainfall from 1st June to 30th November		No. of days of precipitation								Total number of days of precipitation	
			August		September		October		November			
	Nagpur	Akola	Nagpur	Akola	Nagpur	Akola	Nagpur	Akola	Nagpur	Akola	Nagpur	Akola
1928	34·53	26·97	17	10	18	12	8	6	1	..	44	29
1929	40·00	23·12	18	6	16	12	4	2	0	..	38	20
1930	30·51	25·94	18	10	12	11	8	3	3	1	41	25
1931	56·91	41·79	27	27	18	13	15	12	3	5	63	57
1932	44·02	26·49	25	13	14	11	3	2	1	1	43	27

TABLE II.

Months	1928		1929		1930		1931		1932	
	Rainfall in inches	Deviation from normal	Rainfall in inches	Deviation from normal	Rainfall in inches	Deviation from normal	Rainfall in inches	Deviation from normal	Rainfall in inches	Deviation from normal
				Nagpur						
August .	3·29	—7·35	7·07	—3·57	5·95	—8·07	17·59	+5·95	7·49	—4·15
September.	9·65	+1·40	13·58	+5·33	4·63	—3·62	7·42	—0·83	2·69	—5·56
October .	3·43	+1·33	2·48	+0·38	1·44	—0·66	8·02	+6·92	0·97	—1·13
November .	0·00	—0·71	0·00	—0·71	0·11	—0·60	0·34	—0·37	1·53	+0·82
				Akola						
August .	5·45	—0·98	0·38	—6·05	3·83	—2·60	8·98	+2·55	5·39	—1·04
September.	6·72	+1·03	3·31	—2·38	14·76	+9·07	7·11	+1·42	6·74	+1·05
October .	3·31	+1·44	0·39	—1·48	2·35	+0·48	8·03	+6·16	0·92	—0·95
November .	0·00	—0·48	0·00	—0·48	0·41	—0·07	1·67	+1·19	0·13	—0·55
				Khandwa						
August .	5·60	—0·89	2·32	—4·17	5·03	—1·46	12·70	+6·21	5·33	—1·16
September.	1·53	—4·44	3·22	—2·75	6·46	+0·49	4·21	—1·76	6·64	+0·67
October .	1·87	+0·64	0·00	—1·23	1·55	+0·32	9·78	+8·55	1·71	+0·48
November .	0·09	—0·44	0·00	—0·53	0·00	—0·53	0·03	—0·50	0·00	—0·53

NOTE.—The rainfall records given in Table I for Akola were kindly supplied by the Superintendent, Government Experimental Farm, Akola, and the rainfall data given in Table II, and the mean humidity data given in Figure 1 were kindly supplied by the Director General of Observatories, Poona. My thanks are due to these officers for the help they have given.

From Table I it is clear that in 1931 the rainfall was much higher than in the three previous years and in the one followincg year ; the total number of days there was precipitation from August to November is also more than in these four years ; especially in October of 1931 the precipitation was for many more days than in the corresponding month of the other four years. From Table II we see that in three widely separated places like Nagpur, Akola and Khandwa the deviation from normal rainfall in October of 1931 was considerably on the plus side.

The mean humidity in Nagpur, Akola and Khandwa in the months of August to November of the year 1931 was higher than that of the corresponding months of 1928 to 1930 and 1932, especially in October and November (Fig. 1).

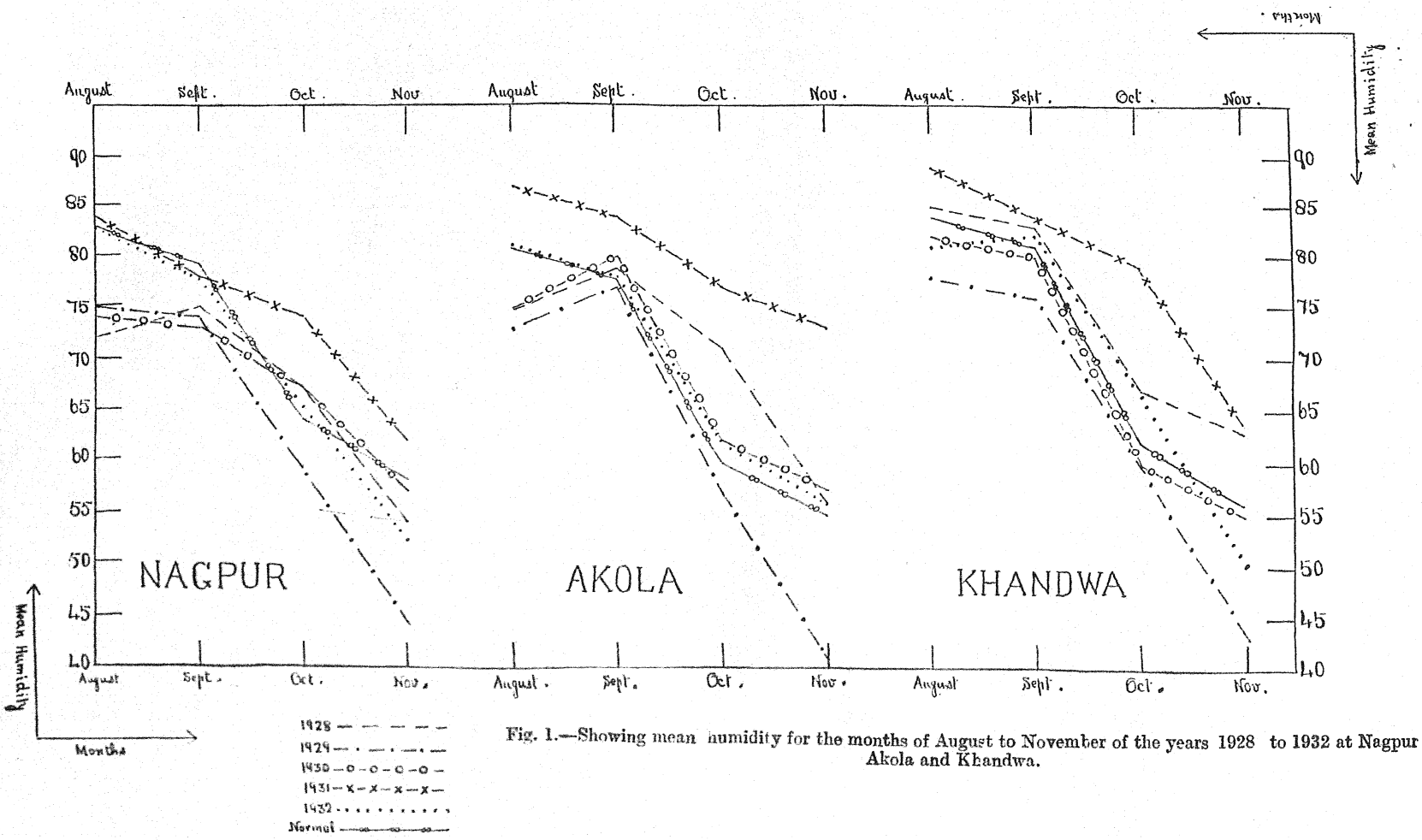


Fig. 1.—Showing mean humidity for the months of August to November of the years 1928 to 1932 at Nagpur Akola and Khandwa.

PLATE XI.



Anthracnose on cotton bolls.

The outbreak of the anthracnose disease in 1931 in an epidemic form is therefore to be attributed to the highly humid atmospheric conditions prevalent in that season, especially in October.

Macroscopic symptoms of the disease.

On bracts, bolls, lint and seed.—On the bracts water-soaked circular spots, brown or black in colour are produced. They are visible on both sides of the bracts; they increase in size rapidly and spread to the boll where the diseased part of the bract is in contact with the boll. When the diseased area extends to the base of the boll, the boll is generally shed. On bracts the pink-coloured oily concentric rings of acervuli have not been observed.

Bolls of all sizes can become infected, from the very minute stud-like bolls to those fully mature. In the early stages of infection the symptoms are not unlike those caused by bacterial blight. The first symptom of the disease is the presence of small circular water-soaked spots which are dark in colour and slightly depressed; they reach a considerable size; the centre turns black and is surrounded by a broad ring of water-soaked area (Plate XI). Usually the central area of the spots is killed; it shrinks and dries up, especially in dry weather. When a very young boll is infected it soon dries up and is shed; in the case of a boll which is infected at a later stage it does not necessarily drop off, before it has dehisced. When the diseased area extends to the lines of dehiscence of the boll, the locks may wholly or partly split open. This may happen even when the boll is small and immature. A badly diseased boll becomes dry or mummified and drops off. When a boll is not badly infected and if all the locks are not diseased, those healthy may continue to grow; the boll thus becomes lop-sided. When mature the lint in the healthy locks bursts through them normally.

When a boll is infected through the pistillary end, the whole boll may become diseased from the tip down to the base; but very often the infection remains confined to its tissues near the tip; the locks split open at the tips and their contents are then found to be rotted.

When the infection penetrates the inner lining of the boll it is stained light yellow in the early stage of infection; but it is ultimately discoloured yellow or brown or even black. It is often covered with minute black dots which are the sclerotia-like stromatic masses of the fungus. The lint hairs in contact with the diseased part of the lining are at first stained yellow ; at a later stage they turn distinctly brown or brownish yellow and in very advanced case they may also become pink coloured.

When a mature boll is badly infected the lint in the diseased lock remains in the cavity of the lobe as a solid compact brittle mass of fibres which cannot be separated

from each other without breaking them; when the boll dehisces this compact mass of fibres either remains enclosed in the lock or drops off as such.

When the lint in a well developed boll is only partially diseased, the diseased portions of the fibres remain cemented together forming a localised compact brittle mass, and the healthy part of the lint fibres open out normally.

When a boll is infected the rapidity with which the diseased area enlarges depends on the prevailing climatic conditions; if the weather is moist or wet the lesions rapidly spread and include all or most of the boll; the brown discoloured areas are covered with oily pink concentric rings of acervuli. In dry weather the development of the lesions is checked, the acervuli are not formed, the diseased area dries up and becomes shrivelled, depressed and woody.

The seed is infected either through the diseased lint hairs or through the placenta. The infection from the lint fibres may not spread to the seed and so after it is ginned the seed is healthy; but if the infection through the lint hairs has extended to the seed the infection remains generally confined to the outer integument of the seed.

But when the infection is direct from the tissues of the boll through the placenta the hyphae from the placental tissues penetrate the funicle and gain entrance inside the seed; in which case the embryo may also become diseased; even if the embryo is not diseased the viability of the seed is affected.

When the seed is ginned or when the lint is removed a diseased seed can be readily distinguished from a healthy one; in severe cases of infection the seed is poorly developed and is light in weight; it may be small in size and have many depressions on its surface; in such seeds the embryo is either aborted or poorly developed. In less severe cases of infection the size and shape of the seed remain normal but the diseased condition of the seed is visible from its colour. A delinted healthy seed is uniformly greyish or greenish in colour due to the presence of fuzz. A diseased seed loses wholly or partly this normal colour which is replaced by different shades of brown or yellow or by brownish yellow. In cases of very slight infection the delinted seed shows irregular small yellowish spots, due to the discolouration of the fuzz. Some parts of the seed are reddish brown or deep brown in colour; this is because the fuzz over a part of the outer integument has flaked off exposing the inner tissues of the outer integument.

On the outside and inside of the seed-coat stromatic masses are developed. These stromatic masses, under moist conditions may grow into acervuli bearing conidia. A seed may be diseased but still it may not show any very visible outward signs of infection. When a diseased seed is cut open, unless it is very badly infected, there are no apparent signs of infection on the inside of the seed.

When a young boll is infected its seeds fail to develop and consequently they are very light and small in size. When the mature boll is badly infected the seed is also diseased it may be of normal size but it is shrivelled and its embryo is entirely rudimentary or dried up. When the seed is slightly infected it is usually plump and capable of germination.

On the seedling.—On the seedling the infection may start from the underground parts or from the cotyledons. Direct infection of the aerial parts has not been observed.

When the disease originates in the basal parts of the herbaceous seedling it shows all the symptoms of a wet-rot or damping off. A water-soaked elongated lesion is first noticeable on one side of the radical or collar, the lesion is reddish or brownish or rusty brown in colour. It increases longitudinally more rapidly than laterally so that the diseased area is over an inch in length before half the circumference of the collar or tap root is discoloured; it looks as if it were pressed on one side giving the stem a half round appearance. At a later stage the diseased part is wholly constricted; when the lesion extends to the surface of the ground or slightly above it the plant usually suddenly collapses, and falls over the soil line. When the lesion is on the part of the tap root near or below the soil surface new roots may be developed from the upper healthy parts of the radical but the lesion rapidly extends upwards, the lower parts being completely decayed, and the seedling is ultimately killed. If however the disease attacks the seedling at a later stage when the stem is becoming woody, the bark at the collar is destroyed and is in shreds, the wood being exposed and discoloured; the lower underground parts show a sort of a combined wet and dry rot but the aerial parts show signs of wilting, they die from top downwards. In either case, whether the seedling shows signs of damping off or of wilting, the cause of the disease can be diagnosed from the presence of small bristly black pin heads which cover the underground parts of the seedling, these are the acervuli. In the absence of the acervuli the damping off or wilting caused by anthracnose cannot be readily differentiated from that caused by *Phythium aphanidermatum* or *Rhizoctonia bataticola* or *R. Solani*. When the seedling, in the herbaceous stage, is affected the chances are that the infection is directly from the seed; but when the seedling shows signs of disease in the woody stage, in all probability the infection is from the soil; in rare cases it is a case of delayed spread of the infection; the seedling was originally infected directly from the seed, but for some reason the infection remained localized and the seedling continued to grow to the woody stage, when the infection which had been so long dormant spread further and killed the bark at the collar.

The infection from the cotyledons is not always fatal. Field observations lead to the conclusion that the infection on the cotyledons is generally direct from the

seed and rarely from air-borne spores. The cotyledons as they emerge from the seed-coat very often show signs of infection. They are not always able to emerge wholly from within the seed-coat, which, when it ultimately drops off carries with it a part of these leaves; the cotyledons at the cut ends have jagged margins, as if a part was torn off or bitten off; the margins are of brown red colour. If the portion of the cotyledons that is left inside the seed-coat be examined, very often it will be found to be covered with the acervuli of the fungus; which if not already formed will soon develop if these torn parts are incubated in a moist chamber. The cotyledons of the the seedling emerging from a diseased seed may be well developed but the signs of infection are to be seen by the presence of one or more circular black water-soaked spots; these may commence from the margins or they may be on any part of the lamina of the cotyledons.

The spread of the disease and the consequent loss of seedlings having infected cotyledons depend on the prevalent climatic conditions; a spell of dry weather checks the further growth of the lesions, the cotyledonous leaf turns yellow and drops off. Thus nature helps in pruning off the infected parts of the seedling. But if the atmospheric humidity is high, the primary lesions on the lamina of the cotyledons extend rapidly to the base of the petiole and involve the tender growing parts and the stem; a soft rot is produced; the diseased parts are brown in colour; and the seedling damps off from top downwards.

On the plant.—After the plant is about two months old and has developed a woody stem, there are very few chances of its being attacked by this disease. However mature dead or dying plants have been found to have their tap-roots covered with acervuli of this fungus and to show a sort of a wet rot where the acervuli have developed; but it is not easy to say if the anthracnose fungus was the original cause of the disease and of the death of the plants or it followed some other disease, like wilt or *Rhizoctonia*. The anthracnose fungus has never been found on the mature woody plant by itself. It has always been found in association with some other diseases of the plant. In the case of a mature woody plant it seems the infection by the anthracnose fungus is only secondary and that it follows in the wake of some other disease.

Microscopic symptoms.

When a discoloured lint hair is examined under the microscope, it is found to be stained yellow and overrun by hyphæ. The hyphæ are both inside and outside the hair. That part of the hair is discoloured where the hyphæ are collected together and form a stromatic mass (Plate XII, fig. 2). The hyphæ bind together in a compact mass a number of hairs, and that is why the lint hairs are not able to open out normally, they remain close to each other as if they were glued together.

PLATE XII.

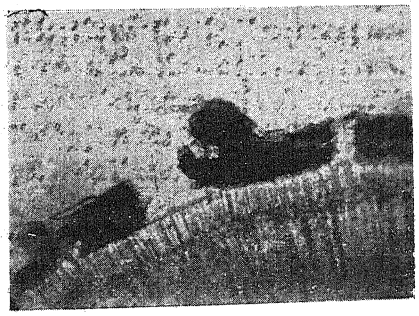


FIG. 1.—Transverse section of a diseased cotton seed showing a sclerotium-like body on the outside of the seed-coat, and the broken outer layer of the outer integument.



FIG. 2.—Infected lint hairs from a diseased boll.



FIG. 3.—Longitudinal section of a diseased seed showing sclerotia-like bodies on the outside and inside the seed-coat. Note the separation of the two layers of the palisade tissue.



FIG. 4.—Longitudinal section of a seed through the funicle showing a collection of hyphae between the funicular tissues and the palisade tissue.

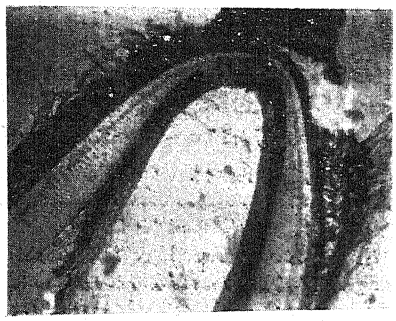


FIG. 5.—Longitudinal sections of a seed through the funicle showing the narrowing of the palisade layers towards the funicular end.

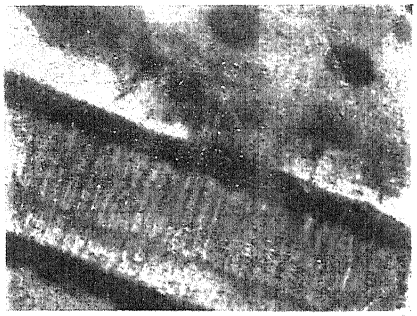


FIG. 6.—Longitudinal section of a diseased seed showing acervuli on the embryo leaf. (The two dark circular spots on the left are leaf glands.)

In sections of diseased seeds the hyphæ are generally observed to be confined to certain parts of the seed. In the outer integument they are found in abundance; the cells are displaced and the hyphæ permeate between the cells (Fig. 2 (*1*), (*3*) and (*4*), they may collect together forming stromatic masses which are composed of brown coloured pseudo-parenchymatous cells. On account of the development of these stromatic masses in the tissues of the outer integument the cells above these stromatic masses are pushed up and as a result in sections the outer layer of the seed-coat has an irregular outline. The stromatic masses are also developed on the upper layer of the outer integument (Fig. 2 (*2*) and Plate XII, fig. 1). The tissues of the outer integument are destroyed by the fungus and so they flake off in parts; in sections therefore the outer integument does not appear to be always continuous (Plate XII, fig. 1). As a rule the mycelium is confined to the tissues of the outer integument; especially when the infection is not very advanced. In some cases the hyphæ have been found in the upper layer of the palisade tissue, here the hyphæ are sparse and always intercellular. The second or the inner layer of the palisade tissue has invariably been found to be free from the presence of hyphæ.

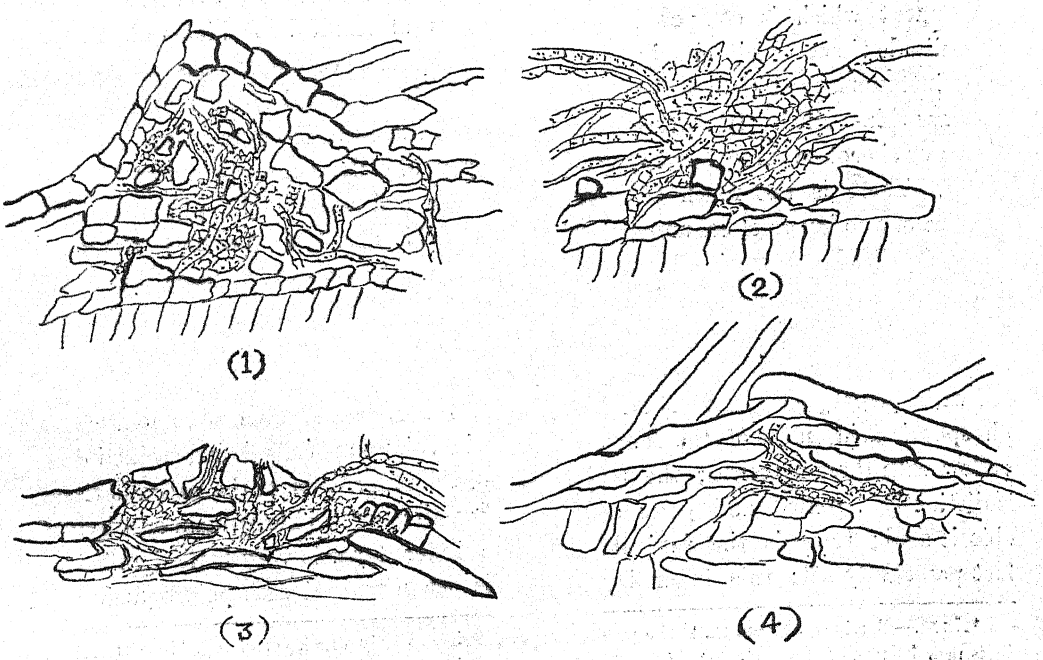


Fig. 2.—(*1*)-(*4*) Sections through the seed-coat of diseased seeds showing the collection of hyphæ in stromatic masses in or on the tissues of the outer integument (×160).

The inner integument may or may not show the presence of the hyphæ. In this tissue the hyphæ are present when the seed is badly infected; the hyphæ overrun the tissues; in advanced cases there is also the formation of acervuli and stromatic masses as in the outer integument (Plate XII, figs. 3 and 6). Ordinarily the hyphæ are found in all the tissues of the seed-coat except the inner layer of the palisade tissue.

The embryo is generally free from infection but in some cases the cotyledonous leaves show the hyphæ on them or in their tissues; in very advanced cases of infection the embryonic tissues are overrun with hyphæ, stromatic masses and acervuli (Plate XII, fig. 6).

There appears to be one layer of cells of the seed-coat which is always free from infection by the hyphæ and still in advanced cases of infection they are present on either side of it. The question therefore naturally arises as to how the hyphæ enter the inner tissues of the seed-coat though these tissues are protected by a belt of the inner layer of the palisade tissue through which the hyphæ cannot penetrate. A study of the anatomy of the seed-coat of a cotton seed would supply the answer.

The seed-coat of a mature seed consists of the following tissues, (1) outer integument which is composed of many layers of small but thick-walled cells, (2) palisade tissue which clearly consists of two layers of columnar cells, and (3) the inner integument, which like the outer integument is composed of layers of cells, but they are thin-walled. In mature seeds the cells of the outer integument are coloured brown or chocolate brown and the different layers of cells cannot be easily differentiated. There is not much of cellulose in their cell walls. The palisade tissue consists of two layers of columnar cells (Plate XII, figs. 1, 3, 5 and 6), the outer layer of palisade tissue is shorter in length than the cells of the inner layer; the difference between these two layers practically vanishes towards the funicular end where the palisade tissue is narrowest in width (Plate XII, fig. 5); the outer layer of the tissue is very slightly lignified and chiefly consists of cellulose; it does not stain red with phloroglucin and hydrochloric acid, but it stains blue with iodine and phosphoric acid, most of the inner layer of the columnar cells being stained deep yellow with these reagents; the major part of the inner layer of the palisade tissue is distinctly lignified and gives a positive reaction with phloroglucin and hydrochloric acid; towards the funicular end the inner layer is very little lignified, in fact practically *nil*; this inner layer of cells when treated with phloroglucin and

*NOTE.—Barritt considers that the palisade layer consists of only one layer of cells [Barritt, 1929]. This may be true of the seed-coat of the Egyptian cotton he studied; but there is no doubt that in the seed of the cotton, Verum 262 (a variety of *Gossypium neglectum*), the palisade layer distinctly consists of two layers of cells; these two layers can be separated from each other by proper manipulation (Plate XII, fig. 3).

hydrochloric acid does not form a complete red ring but the red stained cells are in the shape of a horse shoe ; the unstained cells are toward the funicle, they are thin-walled and very short in length; they take the blue colour with iodine and phosphoric acid.

The inner integument generally consists of cells, the cell walls of which are thin and composed of cellulose; but at times it partly consists of cells which are coloured brown or reddish like those of the outer integument. The differentiation between the several layers of the inner integument is not always very distinct. This integument may be composed of a number of collapsed cells. It is often like a thin membraneous lining, the different cells of which cannot be differentiated from each other. At times the layers of cells adjoining the embryo have completely collapsed and form a reddish brown membrane between the embryo and the seed-coat.

From transverse sections of the seed-coat of a badly diseased seed no evidence can be found as to how the hyphæ are present on either side of the palisade tissue, since this tissue is always free from the presence of hyphæ; but from a study of longitudinal sections it is possible to form a picture of the mode of infection of the inner side of the seed-coat. In longitudinal sections through the funicle it is seen that the tissues of the funicle are completely overrun by hyphæ and that a felt of mycelium is formed between the funicular tissues and the palisade tissues which towards the funicular end consist of thin-walled short cells, and which cannot be always differentiated from the walls of the outer integument (Plate XII, fig. 4); it is through this end that hyphæ have been found to penetrate inside the seed coat. It is thus a selective action on the part of the fungus hyphæ.

Germination of a diseased seed.

From what has been said above it is clear that unless a seed has been infected in the early stages of its development it may be viable even if infected; but this does not necessarily mean that the seed can always grow into a plant.

A badly infected seed as a rule fails to germinate, but in those cases where the embryo is viable the seed may germinate but before the seedling leaves the seed-coat it is liable to be killed, especially under very moist conditions; the radical soon shows signs of the infection and developes a wet rot; the infected part of the radical has a rusty colour and is soft. If the infection on the germinating radical is directly through the mycelium from the seed-coat the seedling is killed before the cotyledons are visible. But if the infection is through spores liberated from the acervuli on the seed-coat the death of the seedling is not so rapid. The cotyledons may emerge from the seed-coat and normal leaves may also be developed before the plant dies from the spread of the infection to the growing point.

Control measures.

Judging from field observations and from the definite connection established between high humidity in the month of October and the incidence of disease on bolls, it seems probable that spraying the bolls as soon as an epidemic of this disease is threatened would considerably check its progress. Whenever there is very high humidity in the month of October, when the first bolls are set, they may be protected against infection with a suitable fungicide. The critical period is therefore of a short duration—of about a month from the end of the rains to the beginning of winter when normally there is a drop in atmospheric humidity—so it is likely that only one application of the fungicide would be sufficient. There has been no opportunity to study the effect of spraying on bolls in an infected field, as last year (1932) the mean atmospheric humidity was not above normal in the month of October.

Since it has been shown that the disease is carried with the seed, that the germination of infected seed is poor and that there is also the danger of carrying the infection from diseased seed and seedlings to healthy seedlings and plants, the second method of controlling the disease seems to be seed treatment.

Many experiments have been done to see the effect of fungicides on the germination of infected seeds. For one of these experiments diseased seeds were selected. These seeds were judged to be diseased by the presence of discoloured spots on the seed. The seeds were plump and normal in size. Except for the discolouration they did not differ from normal seeds. Thirty-six seeds were used for each of the various treatments: they were planted in six pots with six seeds in each. Table III gives the results.

Table III.

Seed treatment	No. of seeds germinated	No. of healthy plants at the end of the experiments
Pickling for 10 minutes in 2 per cent. copper sulphate solution .	6	2
Pickling for 2 hours in 0·25 per cent. uspulun solution . .	12	11
Delinting for ½ hour with sulphuric acid.	11	10
Dusting with copper carbonate	10	5
Dusting with sulphur	12	9
Control	5	1

From this table it is evident that the germination of diseased seeds is very poor and that certain seed treatments are effective in raising the percentage of seed

germination, and that as a result of treatment with some of these fungicides a larger number of healthy plants are obtained even though the seed is diseased.

TABLE IV.

Seed treatment	Seeds germinated		No. of healthy plants alive at the end of experiment	Death of seedlings due to anthracnose		Percentage of plants alive in relationship to the No. of seeds germinated	Percentage of plants alive in relationship to the No. of seeds sown
	No.	Percentage		No.	Percentage		
Pickling in 0·25 per cent. uspulun for 1 hour.	126	70	113	10	8	89·6	62·8
Pickling in 0·25 per cent. uspulun for 2 hours.	120	66·6	109	7	5·5	90·8	60·5
Delinting with sulphuric acid for ½ hour	112	62·2	93	14	11·6	83·0	51·6
Control	115	64·0	98	15	11·0	85·2	56·4
Pickling in 2 per cent. copper sulphate for 10 minutes.	54	30	41	11	20·4	75·9	22·8
Dusting with copper carbonate . .	98	54·4	90	8	8·1	91·8	50·0
Dusting with uspulun . . .	104	57·7	95	8	7·7	91·3	52·0
Heating in sun for 2 hours . .	96	54·0	88	8	8·2	92·7	49·0

TABLE V.

Seed treatment	Seeds germinated		No. of healthy plants alive at the end of the experiment	Death of seedlings due to anthracnose		Percentage of plants alive in relationship to the No. of seeds germinated	Percentage of plants alive in relationship to the No. of seeds sown
	No.	Percentage		No.	Percentage		
Pickling in 0·25 per cent. uspulun for 1 hour.	122	68	60	54	44·3	49·1	33·0
Pickling in 0·25 per cent. uspulun for 2 hours.	111	62	76	32	28·8	68·4	42·2
Delinting with sulphuric acid for ½ hour.	90	50	62	27	30·0	68·8	34·4
Control	101	56	39	58	57·4	38·6	21·6
Pickling in 2 per cent. copper sulphate for 10 minutes.	91	50	61	27	30·0	67·0	33·8
Dusting with copper carbonate.	70	39	56	13	18·6	80·0	31·1
Dusting with uspulun .	101	56	93	6	6·0	92·0	51·6
Heating in sun for 2 hours.	114	63	99	10	8·8	86·8	55·0

Seeds for the experiments, the results of which are given in Tables IV and V, were obtained from the seed stock of the Akola Agricultural Farm; the variety being Verum 262; they were found to be a mixed lot of seed, consisting of healthy and diseased seed; the percentage of diseased seed was 23·3. Seeds which from a naked eye examination were found to be discoloured were regarded as diseased. It is probable that in the lot of "healthy" seeds there may be some diseased ones, those in which the discolouration was not easily noticeable. Tables IV and V give the results of the seeds sown on the 30th June, and 10th August, 1932 respectively; the seeds were sown in an experimental area where cotton was not previously grown. 180 seeds were sown for each of the trials in three rows of 60 seeds each.

TABLE VI.

Seed treatment	Seeds germinated		No. of healthy plants alive at the end of the experiment	Death of seedlings due to anthracnose		Percentage of plants alive in relationship to the No. of seeds germinated	Percentage of plants alive in relationship to the No. of seeds sown
	No.	Percentage		No.	Percentage		
Pickling in 0·25 per cent. uspulun for 1 hour.	556	69·5	526	25	4·5	94·6	65·75
Pickling in 0·25 per cent. uspulun for 2 hours.	511	64·0	493	10	1·9	96·4	61·6
Delinting with sulphuric acid for ½ hour.	399	50·0	380	17	4·2	97·7	47·3
Control . . .	520	65·0	443	69	13·3	85·2	55·4
Pickling in 2 per cent. copper sulphate for 10 minutes.	483	60·3	437	41	8·5	90·4	54·6
Dusting with copper carbonate.	353	44·0	322	26	7·3	91·2	40·25
Dusting with uspulun .	429	53·0	414	15	3·5	96·3	51·75
Heating in sun for 2 hours.	443	55·3	405	30	7·0	91·4	50·5

The seed used for the trials mentioned in Table VI formed part of the stock of the seed that has been used in the experiments recorded in the previous two tables (Tables IV and V). The number of seeds sown for each of these treatments was 800. They were sown by hand in two rows of 400 seeds each on the 10th of August, 1932, on a piece of land on the Nagpur Agricultural College Farm. In many cases the total of the number of plants alive at the end of the experiment is less than the sum of the number of seeds germinated and of the number of seedlings that died of anthracnose, recorded in columns two and four; this difference is due to death of seedlings from other causes than anthracnose, *e.g.* *Rhizoctonia*, *Phytophthora*, etc.

At the Akola Agricultural Farm each of the treatments recorded in Tables IV and V were tried on a field scale. The area under each treatment was 1/40 of an acre. It was not possible to keep a careful count of the percentages of germination, of infection and of healthy plants as recorded in the above tables, therefore soon after the seeds had germinated a thousand seedlings were selected at random in each of the series and they were kept under observation till the 4th of August, 1932. The seeds were sown on the 22nd June. The results obtained by this method were checked on the same day by a second method. In each series three rectangular plots were selected, each plot in a particular series was a fair sample of the conditions prevailing in that particular series. These three plots were separated from each other as far as possible. Each plot covered five rows of plants and was 15 feet in length. The results of these counts are given in Table VII.

TABLE VII.

Treatment	Number of surviving plants out of the 1,000 kept under observation	Number of healthy plants found in the three rectangular plots
Pickling for 1 hour in 0·25 per cent. uspulun* . .	504	656
Pickling for 2 hours in 0·25 per cent. uspulun . .	881	997
Delinting with sulphuric acid for half an hour . .	803	861
Pickling in 2 per cent. copper sulphate solution for 10 minutes.	609	483
Dusting with copper carbonate	721	597
Heating in the sun for two hours	716	711
Dusting with uspulun	804	790
Control	712	706

* This series was situated in a low lying part of the field; and so many seedlings were washed away by the rush of water during heavy precipitations.

NOTE.—These results only show the number of plants alive on the day the counts were made, they give no idea of the cause or causes of the deaths of the seedlings.

At the Agricultural Farm at Khandwa these experiments were also tried. Counts of living healthy plants were made on the 22nd August, 1932, the seeds having been sown on the 29th of June. The number of healthy plants in these equal-sized rectangles, in each series were counted in the same way as described above. The results are given in Table VIII.

TABLE VIII.

Nature of treatment	Number of living plants in three rectangles on 22nd August 1932	
	Roseum	Verum 262
Pickling for 1 hour in 0·25 per cent. uspulun	217	207
Pickling for 2 hours in 0·25 per cent. uspulun	197	195
Delinting with sulphuric acid for ½ hour	225	273
Control	177	137
Pickling in 2 per cent. copper sulphate solution for 10 minutes.	142	199
Dusting with copper carbonate	215	266
Dusting with uspulun	220	209
Heating in the sun for 2 hours	...	177

If we take into consideration the effect of the various treatments tried on germination, we find that when each of these treatments has been repeated the results do not seem to be uniform; the rate of germination differed at times considerably though the seed for the different trials was taken from the same stock of seed; *e.g.*, if we refer to Tables III, IV and V, we see that the percentages of germination of the "control" or untreated seed have been 64, 56 and 65; of those pickled in copper sulphate 30, 50 and 60; of those dusted with copper carbonate 54, 56 and 44; of those heated in the sun 54, 63 and 55; these variations may be due to the fact that the number of diseased and healthy seeds in each lot of seed taken from the stock would vary considerably from one another; in one lot there may be many more diseased seeds than in another lot. This variation would be particularly more so in small lots of seeds, say of a hundred each. Therefore in considering the effects of the various treatments tried much importance need not be given to the rate of germination; however it is evident that seeds pickled in uspulun have given in a majority of cases a higher rate of germination and that seeds treated with copper sulphate have uniformly given a lower rate of germination. If we consider the effects of the various treatments on the seedlings developed from these treated seeds we find that the percentage of anthracnose on seedlings from most of the treated series is less than the percentage of anthracnose on seedlings raised from untreated seeds. If we take the results of treated seed sown on a large scale, at Akola and Khandwa, we find that the seeds either treated with uspulun, dry or wet, or delinted with sulphuric acid have given a larger number of healthy plants; and these two treatments have given the best results. Seeds dusted with copper carbonate have also given a larger number of healthy plants than the untreated seeds. Pickling in copper sulphate has been found to affect considerably the germination. Heating the seed in the sun for two hours has not

uniformly been successful in giving a larger number of healthy plants than the untreated seeds.

Treating diseased seed with uspulun either by the wet method or by the dry method can be recommended; but unfortunately this proprietary fungicide is now not manufactured because according to the manufacturers they have replaced it with a better fungicide called ' ceresan '.

Delinting with sulphuric acid cannot be recommended to the cultivators because of the obvious difficulties involved in the use of this vitriolic acid, even though not only are seed-borne diseases of cotton controlled by treating diseased seeds but there are also other advantages. Delinted seeds germinate more quickly, and therefore the danger of their being susceptible to seedling blight, caused by *Rhizoctonia bataticola* (Taub.) Butl., is reduced. It has already been shown that delayed germination is favourable for infection by seedling blight [Dastur, 1931]. Secondly by removal of lint and fuzz the seed can easily run through a sowing drill and therefore the necessity of coating seeds with a cow-dung solution, as is the general practice in the case of fuzzy seeds to facilitate their passing through the drill, is obviated.

The following method of delinting seed with sulphuric acid was adopted. One part by volume of commercial sulphuric acid was used to delint 15 to 20 parts by volume of the seeds. The seeds to be delinted were placed in a glass or porcelain vessel or a wooden container, the inside of which was coated with hot melted pitch; the acid was slowly added; the seeds were continuously stirred by means of a wooden or bamboo stick till all the seeds were well covered with the acid; they were allowed to remain in contact with the acid for 20 to 30 minutes. At the end of this period the seeds were thoroughly washed in a very large quantity of water; it is essential that the quantity of water should be very large so that the heat generated by water coming in contact with the acid is not high enough to scorch or damage the seeds. It was found advantageous to add lime to the water used for washing the acid-coated seeds. The washed seeds were spread out in a room to dry. Dried delinted seeds germinated normally even when stored for two months before sowing.

Inoculation experiments.

Bolls of different sizes were inoculated with spore suspensions, either through needle punctures or through the unbroken skin. The infection took more rapidly when the inoculation was either through a puncture or on the suture of two adjoining loculi. The characteristic water-soaked, depressed, circular lesions were visible within two days after the inoculation. The lesion increased in area and penetrated through the walls of the boll to the enclosed lint or its progress was checked and it

dried up according as the climatic conditions were wet or dry. If the inoculated boll was enclosed in a glass chimney, plugged with cotton wool at both ends so as to ensure high humidity, the infection spread rapidly, the whole boll being involved within four or five days, and the characteristic pink acervuli in concentric rings were developed; when the lesion reached a suture or when the inoculation was done on the suture the adjoining loculi split open and the exposed lint was infected rapidly. When the inoculation was through the tip all the locks split open in that region. Bolls of all ages and sizes could be inoculated provided high humidity was maintained.

Seedlings in the herbacious stage have been successfully inoculated by placing the inoculum near the collar or on the tap-root below the soil surface. The inoculated seedlings have shown the typical damping off symptoms within four or five days after they were inoculated. Seedlings with a woody stem have not always been successfully inoculated except when the collar or the tap-root was wounded before placing the inoculum on them; plants with a well developed woody stem have failed to take the infection even through wounds. Flowers and flower buds took the inoculation rapidly. Infected flower buds dropped off without opening; inoculated flowers were either shed or only the corolla dropped exposing the button-like small boll which showed signs of infection at the pistillary end which was slightly depressed. These tiny bolls soon dropped off before they were many days old.

Inoculations of chilli fruits, tomato fruits and sugarcane leaves, even through wounds with this fungus were not successful.

Microscopic characters of the fungus.

The bristly black raised acervuli, that are seen scattered on the infected parts of the stem of seedlings and the pink coloured oily globular acervuli aggregated together and arranged in compact concentric rings on the infected boll, consist of basal stromatic tubercles which are composed of a number of brown coloured pseudo-parenchymatous cells; the surface cells of the tubercles develop into a number of erect, short and narrow cells closely pressed together like so many columns arranged in rows (Fig. 3); they are generally hyaline in colour, but at times they may be sub-hyaline; they are finger shaped, broadly rounded at the apex and flat at the base; very often they are slightly curved; they measure 7·7-13·2 $\times$ 1·6-2·7 μ; from the tips of these cells, which are the conidiophores, falcate shaped conidia are budded off; at first a slight constriction a little below the apex is developed; this dot-like apical constricted part is the conidium in making; it is at first continuous with the conidiophore from which it has developed; it grows in size and becomes elliptical or ellipsoidal in shape and cut off from the conidiophore by the constriction growing inwards and forming a septum. When mature

the conidium becomes falcate in shape and readily drops off. A succession of spores may be developed from the tip of one conidiophore; thus the formation of conidia is acrogenous. The mature conidia are falcate in shape and are either sharply pointed at both ends or only at one end, the basal extremity, the other being rounded. They are hyaline in colour and have one or two vacuoles; they germinate by putting out a germ tube generally only from one end, occasionally germ tubes may develop from both ends. While germinating the shape of the spore may be slightly altered, it may become broader and also septate. Appressoria are generally formed terminally or laterally from the germ tube. The conidia measure usually 20·0-22·5 ⩒ 2·5 μ, the extreme measurements being 15·0-25·0 ⩒ 1·8-4·3 μ.

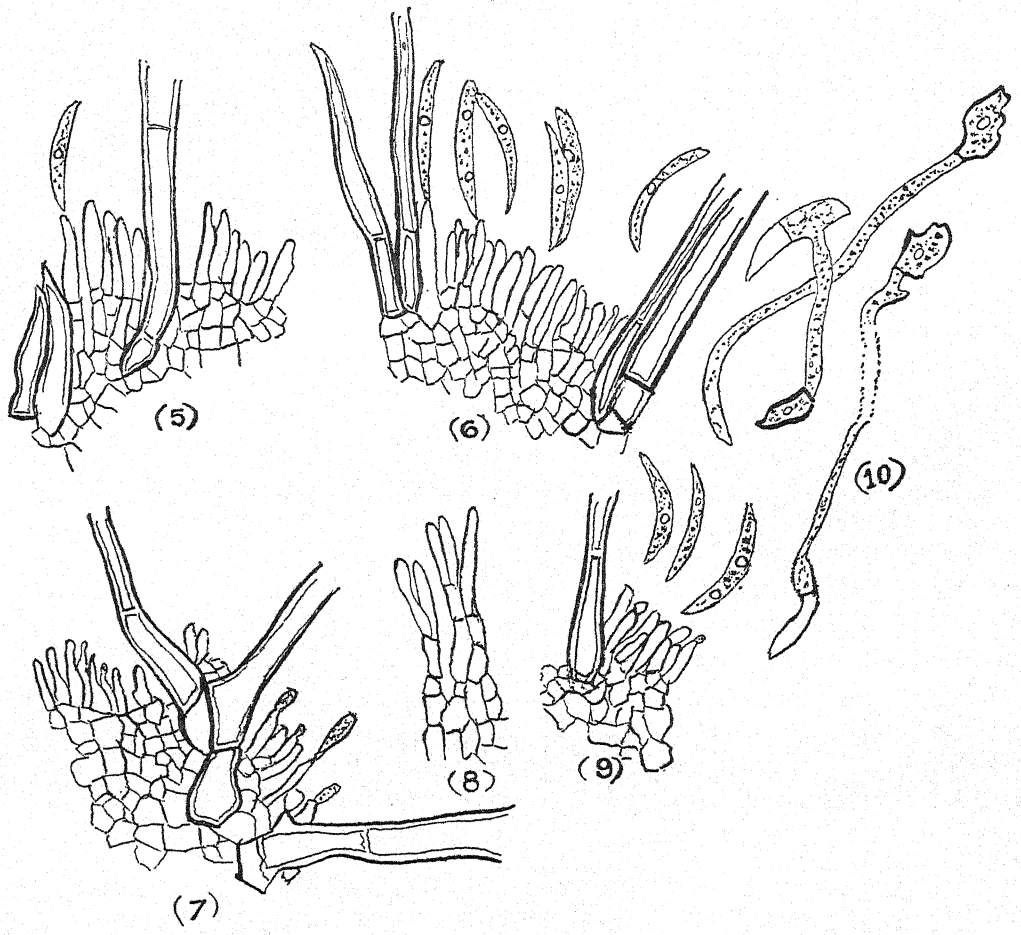


Fig. 3 :—(*5*)-(*9*).—Sections of acervuli showing pseudoparenchymatus cells, conidiophores, conidia and setae (×720); (*10*).—Germinating conidia (×720).

Interspersed with the conidiophores are thick-walled setae, they develop from the basal stromatic cells. They are commonly many-celled. The number of septa usually vary from 0 to 7. The setae are dark brown in colour but the basal and apical cells are often much lighter coloured. They are not necessarily rounded at the base. They are sharply pointed or the apex may be obtuse angled. They measure 76·5-2·55✓3·8-7·6 μ.

The fungus is provisionally named *Colletotricum indicum* Dast.

Susceptibility.

It seems from field observations that Verum 262 is more susceptible than Roseum to anthracnose. These observations have been confirmed by examining the seed from the first picking of these two varieties. Various samples were examined with the naked eye. Those that showed even the slightest discolouration on the fuzz or on the seed coat were considered to be infected by anthracnose; those that were found to be normal in colour were classed as healthy; it is not improbable that some of these latter may have been diseased, the discolouration being not noticeable with the naked eye. The percentage of diseased seed in samples of the first picking of Verum 262 and Roseum obtained from the experimental area of the Economic Botanist for Cotton at Nagpur were 15·5 and 1·5, respectively; the percentage of diseased seed in samples of the first picking of Verum 262 and Roseum obtained from the Agricultural Farm, Akola, was 12·5 and 3·3 respectively.

In 1932, during the first few weeks after germination the death rate of seedlings was very high, not on account of the incidence of seedling blight caused by *Rhizoctonia bataticola*, and *Pythium aphenidermatum* which was practically negligible, as the weather conditions at the time of sowing were very favourable to germination; but in a large majority of cases the death of seedlings was found to have been due to anthracnose. We have seen that from anthracnose-infected seed diseased seedlings are generally produced which usually die before they make any further growth. Therefore the number of healthy plants about eight weeks after sowing in a given area would be a fair indication of the quality of the seed used which in turn would show the susceptibility to anthracnose of the variety to which the seed belongs. If counts were made of the herbaceous seedlings in the stage when no more deaths from anthracnose were to be expected and before the plants became susceptible to wilt a fairly accurate indication of the relative susceptibility of different varieties could be had, provided varieties with the same sowing seed rate were selected for comparison and the area in which the counts were made was the same in each case.

Counts were therefore made at the Agricultural Farm at Akola on the 5th August, 1932. The following varieties having the same seed rate and which were grown on an extensive scale on the Farm, were selected ; Bani, Roseum, E. B. No. 31 (Bani Selection), Verum 262, Late Verum and Early Verum. Three equal-sized plots in fields of each of these varieties were marked out ; each of these three plots showed the average condition of the field in which they were ; each plot covered five rows of plants and was 15 feet in length.

The results of these counts are given in Table IX.

TABLE IX.

Variety	No. of healthy plants on the 5th August 1932
Bani	592
Roseum	456
E. B. No. 31 (Bani Selection)	399
Verum 262	219
Late Verum	201
Early Verum	155

Buri is very resistant to anthracnose but is not taken into consideration as the seed is much larger than the seeds of the varieties mentioned above and therefore the seed rate is necessarily smaller ; so the number of plants in a given area is much less than those of the other varieties in the same area.

From Table IX it is clear that Bani is most resistant to anthracnose, Roseum is second best and the Verums are very much susceptible.

In 1931 when there was an outbreak of the anthracnose epidemic different varieties of cotton grown by the Economic Botanist for Cotton at Nagpur were carefully examined on the 2nd November 1931 to see if there was any difference in susceptibility to anthracnose amongst these varieties. Counts of diseased bolls on plants in four rows, each 10 ft. in length, of some of the varieties were made.

The results are given in Table X.

TABLE X.

Variety	No. of diseased bolls
G. N. Verum ordinary	46
„ „ „ 535	46
„ „ „ 262	56
„ „ „ 264	55
„ „ „ 265	40
„ „ „ 2169	50
G. indicum Bani 306	19
G. N. Roseum	24

These results also show that the different varieties of Verum are more susceptible than Roseum and that Bani is better than either of these two. These results are in accord with those recorded in Table IX.

SUMMARY.

1. A new anthracnose disease of cotton bolls and cotton seedlings is described.

2. This disease is different from the one known in America and some other places, where it is caused by *Glomerella gossypii* (Southw.) Edg.

3. Evidence is produced to show that the cause of the epidemic in 1931 was due to high atmospheric humidity in October of the year.

4. The disease is carried in the seed.

5. Microscopic characters of an infected seed are described in detail.

6. Hyphæ are found inside the seed-coat and it is suggested that they get there through the funicular end.

7. Germination of diseased seeds is found to be poor.

8. Seedlings from infected seeds are liable to be killed by the fungus from the diseased seed.

9. Remedial measures found effective in raising the percentage of healthy seedlings from diseased seed are treating the seed with uspulun, sulphuric acid or copper carbonate.

10. The fungus is provisionally named *Colletotricum indicum* Dast.

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MOVEMENT OF SULPHATE OF AMMONIA WHEN ADDED AS A FERTILIZER TO SOILS.

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I. Introduction.

During the experiments in manuring *rabi* crops at the Poona Agricultural College Farm it was observed that when sulphate of ammonia was sown between two rows of *rabi jowar* 18 inches apart, it did not show any effect on the crop. This led to the suspicion that perhaps the fertilizer did not move enough to be within the feeding zone of the roots of the crop. It was therefore decided to carry on definite experiments on the movement of sulphate of ammonia in the soil. The experiments were chiefly carried out in the laboratory with typical soils of the Bombay Presidency under varying conditions of moisture. These were then confirmed by experiments in the field and by the study of the root system of the *jowar* plant.

Any soluble fertilizer is likely to be moved in the soil as follows :—

(1) The fertilizer may move with the water which flows from that part which has a higher proportion of water to another part which has a lower proportion of water. This movement may be in any direction. (2) The fertilizer may move only towards the surface of the soil if the capillary action due to evaporation at the surface pulls the fertilizer in that direction and (3) lastly the fertilizer may move due to diffusion of the salt without any reference to the actual movement of the water itself. Since the experiments were to be studied under *rabi* conditions when moisture is evenly distributed in the soil, the question of the movement of the fertilizer with the actual moving currents of water was not of great importance. The capillary action will bring the fertilizer only towards the surface and that therefore does not give any idea as to how the fertilizer will move to be within the feeding zone of the roots of the plants. It is the study of the diffusion of the fertilizer that is really important to give a solution of the question about the movement of fertilizers to be within the feeding zone of the roots. In the experiments described in this paper movements of sulphate of ammonia due to diffusion and also due to capillary action were studied.

Hall [1909] refers to the diffusion of salts in the soils at Rothamsted and says :—

"Of the extreme slowness of the diffusion of the soluble salts in the soil, Rothamsted Experiments are some good examples. For instance on the grass plots only an imaginary line divides the plots receiving different fertilizers. ..... Although the treatment has been repeated now (1905) for over 52 years the dividing line between the plots remains sharp and the rank vegetation produced by the excess of the nitrogenous fertilizer on one side does not stray more than 6 inches over the boundary."

Brown [1928] speaking about the movement of soluble nitrogenous fertilizers says that some horizontal movement may take place but it is negligible in comparison with the capillary and gravitational movement.

Cook [1916] worked on the retention and distribution of sulphate of ammonia. He filled paper tubes with soils containing varying proportions of water and poured sulphate of ammonia solution on the top. After three days he cut the tubes into sections and analysed the soils. He found that ammonia quickly moves down to lower layers in light soils not rich in lime but in heavy soils most of the ammonia is held in the surface three or four inches. The experience of these and other workers shows that the movement of sulphate of ammonia in the soils is slow and the actual rate will vary according to the nature of the soil.

For the purpose of this paper three typical soils of the Bombay Presidency were taken. They were (1) Medium black soil derived from trap, Poona District, (2) Laterite soil, Belgaum District, and (3) Alluvial *Goradu* soil, Kaira District. The exact nature of these soils will be clear from the following data.

TABLE I.

Mechanical analysis by the sedimentation method.

Fraction	Medium black soil	Laterite soil	*Goradu* soil
	Percentage	Percentage	Percentage
Clay and finest silt (Below 0·001 to 0·002 mm.) .	24·80	36·46	10·84
Fine silt (0·002 to 0·01 mm.)	30·71	4·48	5·20
Medium silt (0·01 to 0·03 mm.) . . .	10·52	8·37	5·85
Coarse silt (0·03 to 0·07 mm.)	13·50	13·54	24·49
Fine sand (0·07 to 0·2 mm.) . . .	9·60	19·81	52·04
Coarse sand (0·2 to 1·0 mm.)	10·40	18·43	1·72

The soils were not given any pre-treatment with chemicals.

1. Maximum water holding capacity—

Medium black soil	Laterite soil	*Goradu* soil
Percentage	Percentage	Percentage
48	38	20

2. Sticky point (moisture at—)

Medium black soil	Laterite soil	*Goradu* soil
Percentage	Percentage	Percentage
26·4	16·7	6·4

3. Capillary rise of water is shown by the figures given below. The *Goradu* soil shows quick rise in the beginning but ultimately the medium black soil shows the highest capillary rise followed by the laterite soil, and the *Goradu* soil stands last.

TABLE II.

Capillary rise of water in cm.

Time	Medium black soil	Laterite soil	*Goradu* soil
3 hours	2·5	7·5	12·1
6 hours	5·0	11·1	19·0
12 hours	10·2	19·3	26·6
24 hours	19·5	31·2	43·0
2 days	30·2	46·5	64·0
3d ays	42·4	59·8	74·5
4da ys	55·1	70·2	77·5
5days	70·1	80·1	80·2
6days	85·5	85·2	81·7
7days	96·1	91·3	82·3
8 days	106·2	97·5	83·8
9 days	114·9	102·0	85·0
10 days	125·2	103·2	85·1

4. The percentage loss on ignition and lime contents of the soils are as given below.

	Medium black soil	Laterite soil	*Goradu* soil
Loss on ignition	11·49	8·35	4·30
Lime (CaO)	6·15	0·17	1·42

II. General plan of the experiments.

The following arrangement was found to be convenient in measuring the movement of sulphate of ammonia, horizontally and vertically both by diffusion and by capillary action in typical soils under varying moisture conditions.

A set of brass boxes measuring 4 in. × 4 in. × 24 in. were prepared. At the middle part of the length of each box, two grooves were made into which two brass plates could be slid leaving a clear space of $\frac{1}{4}$th inch between them.

The soil was prepared with the necessary quantity of water and was packed evenly in the box on two sides of the plates, care being taken not to allow any soil to get into the narrow space between the plates. Even packing was ensured by putting equal weights of soils in equal volumes of the box. When the box was filled with soil except the top $\frac{1}{8}$th inch, it was covered with tough brown paper with a slit $\frac{1}{4}$th inch wide just over the space between the two brass plates. The space was then well packed with powdered sulphate of ammonia. The brown paper was removed and the brass plates were drawn out, more soil was put on and an even surface was obtained. The soil was covered with a thin layer of paraffin wax to prevent movements of water due to evaporation at the surface. For each long box of the description given above there was one small box 4 in. × 4 in. × 9 in. into which the same soil as above with the same proportion of moisture was packed and covered with wax. This acted as the blank or check. There were six sets of boxes with blank for each proportion of moisture. At the end of each week one long box and a blank were opened. The soil in the long box was cut into 3 inches blocks, the measurement starting $\frac{3}{4}$th inch from the centre thus avoiding the actual solid sulphate of ammonia coming in. The blocks on two sides at equal distances from the central space were mixed together and analysed. The figures obtained for different blocks were compared with the check.

Such was the arrangement to measure the horizontal movement. For measuring the vertical movement, brass towers were prepared with rings one above the other in such a way that a block of clear 3 inches depth could be obtained by removing each ring. The bottom ring had a bottom to hold the soil. For measuring the upward movement, the sulphate of ammonia was put at the bottom of the lowest

ring and for downward movement it was put at the top of the top ring. In both the cases the top of the soil was covered with paraffin. The checks were of the same type as those given under horizontal movement.

In studying capillary movement no wax was put on the top of the soil thus allowing free evaporation and movement of water towards the surface. Each of the three soils was tried with moistures equal to 25 per cent., 50 per cent., 75 per cent. of its maximum water-holding capacity. Each block was analysed for (1) moisture, and (2) nitrogen.

Nitrogen was determined as follows :—

(*a*) Total nitrogen was determined by Kjeldhal method to include nitric nitrogen by using a mixture of salicylic acid and sulphuric acid with sodium thiosulphate and anhydrous sodium sulphate added [Official and Tentative Methods of Analysis, 1921].

(*b*) Ammoniacal nitrogen was determined by extracting soil with sodium chloride solution as suggested by McLean and Robinson [1924] and distilling the filtered extract over magnesia.

(*c*) Nitrites were determined by Griess Ilosway method and the nitrates by the phenol-disulphonic acid method. In both cases standardized tintometer glasses were used for comparing the developed colours.

III. Horizontal movement of sulphate of ammonia.

Soils were packed in long boxes as described above and one box of each type with a check was opened every week. The soil was cut into blocks of 3 inch length measured from where sulphate of ammonia was put and analysed. The total nitrogen which includes organic, ammoniacal, nitrous and nitric nitrogen is taken as the indication of the movement of sulphate of ammonia. The medium black soil with moisture equal to 25 per cent. of the maximum water-holding capacity gave the following results :—

Table III.

Horizontal movement of ammonium sulphate. Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil.

Time	Distance from where sulphate of ammonia powder was put		Blank
	0 in.—3 in.	3 in.—6 in.	
1 week	174	171	171
2 weeks	252	170	171
3 weeks	317	170	170
4 weeks	367	172	171
5 weeks	410	171	170
6 weeks	448	170	171

In the first three inches there is an increase of total nitrogen week after week, but beyond three inches the total nitrogen is just the same as in the check. This shows that sulphate of ammonia does not diffuse beyond three inches in six weeks in the medium black soil containing moisture equal to 25 per cent. of the maximum water-holding capacity.

With higher proportion of water the same soil gives the following result :—

TABLE IV.

Moisture equal to 50 per cent. of the maximum water-holding capacity. Amount in mgrms. of the total nitrogen per 100 grms. of oven-dry soil.

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
1 week	188	170	171	171
2 weeks	318	206	171	170
3 weeks	430	211	170	170
4 weeks	523	209	170	170
5 weeks	597	213	171	170
6 weeks	643	212	170	170

TABLE V.

Moisture equal to 75 per cent. of the maximum water-holding capacity. Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil.

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
1 week	230	171	170	171
2 weeks	403	240	170	171
3 weeks	553	291	171	170
4 weeks	685	336	170	169
5 weeks	801	373	171	171
6 weeks	911	408	170	170

The figures in the above tables show that sulphate of ammonia moves through small distances by diffusion. With moisture equal to 25 per cent. of the water-holding capacity it does not go beyond 3 inches in 6 weeks and even with 75 per cent. it goes only up to 6 inches. With higher proportion of water larger quantities of the salt are moved. Proportionately a large quantity moves in the first week and as the time advances the quantities moved every week become smaller which means that the rate of diffusion slowly falls off.

Taking Table IV the quantities moved every week are as given below :—

TABLE VI.

Time	Milligrams of total nitrogen present	Increase over previous week
1 week	188	...
2 weeks	318	130
3 weeks	430	112
4 weeks	523	93
5 weeks	597	74
6 weeks	643	46

The experiments on the diffusion of sulphate of ammonia in laterite soil gave results similar to those with the medium black soil. The actual figures obtained with three different proportions of water are given below :—

TABLE VII.

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil. (With moisture equal to 25 per cent. of the maximum water-holding capacity.)

Time	Distance from where sulphate of ammonia was put		Blank
	0 in.—3 in.	3 in.—6 in.	
1 week	124	117	117
2 weeks	162	118	118
3 weeks	192	117	118
4 weeks	219	117	117
5 weeks	237	116	117
6 weeks	254	117	118

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil. (With moisture equal to 50 per cent. of the maximum water-holding capacity.)

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
1 week	154	118	117	118
2 weeks	234	121	118	116
3 weeks	305	118	118	118
4 weeks	368	120	117	118
5 weeks	422	123	118	117
6 weeks	469	127	118	116

Amount in mgrms. of nitrogen per 100 grms. of oven-dry soil. (With moisture equal to 75 per cent. of the maximum water-holding capacity.)

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
1 week	171	123	117	118
2 weeks	303	178	117	117
3 weeks	408	219	118	116
4 weeks	511	253	117	117
5 weeks	673	280	118	118
6 weeks	738	297	118	118

In the laterite soil as in the medium black, the diffusion of sulphate of ammonia does not go beyond three inches with moisture equal to 25 per cent. of the maximum water-holding capacity of the soil. With 50 per cent., the diffusion goes up to six inches at the most but the quantity diffused beyond three inches in six weeks is small. With 75 per cent. the quantities diffused are large but even here the salt is not diffused beyond six inches in six weeks.

Goradu soil is a typical soil of Ahmedabad and Kaira districts and was therefore used to see how far it confirmed the results indicated by the other two soils. The following table shows the quantity of sulphate of ammonia diffused with three different proportions of water in the *Goradu* soil.

TABLE VIII

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil. (With moisture equal to 25 per cent. of the maximum water-holding capacity.)

Time	Distance from where sulphate of ammonia was put		Blank
	0 in.—3 in.	3 in.—6 in.	
1 week	55	49	49
2 weeks	84	48	48
3 weeks	105	47	48
4 weeks	118	48	49
5 weeks	127	47	48
6 weeks	132	46	47

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil. (With moisture equal to 50 per cent. of the maximum water-holding capacity).

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
1 week	62	48	49	47
2 weeks	117	47	47	48
3 weeks	155	48	47	48
4 weeks	188	62	48	49
5 weeks	221	68	47	48
6 weeks	244	65	46	47

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil (With moisture equal to 75 per cent. of maximum water-holding capacity).

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
1 week	89	48	46	48
2 weeks	191	93	47	48
3 weeks	275	117	49	47
4 weeks	347	140	48	47
5 weeks	379	166	47	47
6 weeks	394	187	48	49

From the results given so far it is clear that the larger the quantity of water present in the soil the larger is the quantity of sulphate of ammonia diffused. No definite correlation can be shown between any "single value" of the soil and the quantity of sulphate of ammonia diffused. This is but natural as a soil is a complex combination of "single values" many of which are likely to have their influence on the diffusion of salts. If the quantity of total nitrogen found in the blank is deducted from that found after diffusion of sulphate of ammonia for six weeks in all the three soils with varying proportions of water the following figures are obtained. The maximum water holding capacity of the soils is given to show to what extent roughly the diffusion of salts is influenced by this "single value".

TABLE IX.

Amount in mgrms. of nitrogen per 100 grms. of oven-dry soil. Total nitrogen diffused into the soil at the end of six weeks.

—	Medium black soil	Laterite soil	*Goradu* soil	Proportion to each other		
				Medium Black soil	Laterite soil	*Goradu* soil
	1	2	3	1	2	3
Milligrammes diffused with water equal to 25 per cent. of water holding capacity.	277	137	84	100	49	30
With water equal to 50 per cent. of water holding capacity.	472	352	196	100	74	41
With water equal to 75 per cent. of water holding capacity.	740	621	346	100	83	46
Maximum water holding capacity.	48 per cent.	38 per cent.	20per cent.	100	79	42

With water equal to 50 per cent. of the water holding capacity the sulphate of ammonia diffused in different soils is proportional to the quantity of water present in the soils but with lower or higher quantities this proportion does not hold good. With smaller quantities of water, soils with low water holding capacity show less capacity for diffusion and with larger quantities greater capacity for diffusion than when they contain water equal to 50 per cent. of the water holding capacity.

IV. Vertical movement of sulphate of ammonia.

It is a well known fact that diffusion takes place equally along the radii of a sphere but it was necessary to ascertain how far this holds good with sulphate of ammonia when used on soils under experiment. Observations were taken for diffusion vertically downwards and also upwards.

In these experiments towers of brass rings were fitted with soil as already described and sulphate of ammonia was put on the top of the soil for downward diffusions and at the bottom for upward diffusion. In both cases the surface was covered with paraffin wax. The soils were the same as for horizontal diffusion. Each set of experiment was carried through four weeks. There were therefore four towers and four checks for each set. The towers were cut into three inch lengths and the soils were analysed as for horizontal diffusion. The following results were obtained for the medium black soil for downward diffusion.

Table X.

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil.

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
Water equal to 25 per cent. of water holding capacity—				
1 week	170	169	169	168
2 weeks	248	168	168	169
3 weeks	320	167	167	167
4 weeks	370	169	170	168
Water equal to 50 per cent. of water holding capacity—				
1 week	188	176	169	169
2 weeks	322	207	168	168
3 weeks	436	210	169	168
4 weeks	535	211	168	167
Water equal to 75 per cent. of water holding capacity—				
1 week	234	175	169	168
2 weeks	428	244	168	169
3 weeks	607	301	168	169
4 weeks	743	351	168	168

For upward diffusion in medium black soil with water equal to 75 per cent. of water holding capacity the figures obtained were as follows :—

TABLE XI.

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil.

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
1 week	207	168	168	169
2 weeks	385	215	167	169
3 weeks	498	248	168	169
4 weeks	588	287	168	169

Diffusion vertically upwards or downwards is very similar to that in the horizontal direction, the only difference observed is that in the downward direction there is more diffusion and in the upward direction there is less diffusion than along the horizontal direction. The following figures indicate the actual excess of diffused nitrogen over the blank in the medium black soil with water equal to 75 per cent. of the maximum water holding capacity in three directions.

TABLE XII.

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil.

Time	Horizontal diffusion		Vertically downward diffusion		Vertically upward diffusion	
	0 in.—3 in.	3 in.—6 in.	0 in.—3 in.	3 in.—6 in.	0 in.—3 in.	3 in.—6 in.
1 week	59	0	65	7	38	0
2 weeks	232	70	258	75	197	47
3 weeks	383	113	430	122	330	80
4 weeks	516	168	572	183	419	118

The results obtained with the Laterite and the *Goradu* soils are exactly along the same direction. The figures of the excess of total nitrogen in soils containing water equal to 75 per cent. of the maximum water holding capacity over the blanks for the three directions are given below :—

TABLE XIII.

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil.

Time	Horizontal diffusion		Vertically downward diffusion		Vertically upward diffusion	
	0 in.—3 in.	3 in.—6 in.	0 in.—3 in.	3 in.—6 in.	0 in.—3 in.	3in.—6 in.
Laterite soil						
1 week	53	5	59	9	30	0
2 weeks	186	61	206	68	163	50
3 weeks	292	103	325	114	265	88
4 weeks	394	136	442	142	354	132
Goradu soil						
1 week	41	0	53	4	26	0
2 weeks	143	45	168	66	113	0
3 weeks	228	70	265	30	189	6
4 weeks	296	93	342	62	251	22

V. MOVEMENT OF SULPHATE OF AMMONIA UNDER CAPILLARY ACTION.

The experiments described so far were with soils containing evenly distributed moisture and the movement of moisture was checked by putting paraffin wax on the top of the soil. The study of the diffusion of sulphate of ammonia under, so to say, a statical condition of water is only one aspect of the problem. Water in the *rabi* condition of the soil is sure to have some movement due to evaporation of water at the surface or from deeper portions due to cracks. It is therefore important to know how sulphate of ammonia diffuses when soil loses water at the surface and thus causes a pull on the water below the surface. In these experiments brass rings were fitted into each other to form a tower of one foot in height,

sulphate of ammonia was put at the bottom and soil with a definite proportion of water was filled in and there was no paraffin wax or any substance at the top to prevent evaporation. It was noticed that the top layer soon lost moisture and became hard on the surface in the case of medium black soil but in the case of lighter soils the top layer became quite loose. The results obtained with the medium black soil are given below :—

TABLE XIV.

Amount in mgrms. of total nitrogen per 100 grms. of oven-dry soil.

Time	Distance from where sulphate of ammonia was put			Blank
	0 in.—3 in.	3 in.—6 in.	6 in.—9 in.	
With water equal to 25 per cent. of water-holding capacity.				
1 week	181	167	168	167
2 weeks	263	168	168	168
3 weeks	269	167	168	168
4 weeks	267	168	168	168
With water equal to 75 per cent. of water-holding capacity.				
1 week	345	236	170	168
2 weeks	482	416	168	168
3 weeks	572	596	167	168
4 weeks	653	708	167	168

These figures indicate that in the medium black soil sulphate of ammonia rises and accumulates about six inches below the surface since the top layer gets dry and the capillary connection is broken off. Very similar behaviour is shown in Laterite and *Goradu* soils.

VI. FORMATION OF NITRATES.

The increase in the total nitrogen in different layers was due naturally to the sulphate of ammonia that was introduced to see how it diffused. During the diffusion ammoniacal nitrogen is likely to be turned into another form. The

change into the nitrate form is important from the point of view of the crop to which sulphate of ammonia is used as a manure. In the case of the medium black soil nitrite and nitrate nitrogen was determined. Since nitrite nitrogen was present in very small quantities it is put together with nitrate nitrogen and the two have been shown under one heading " nitric nitrogen ". In the cases where the soil contained water equal to 25 per cent. or 50 per cent. of the water holding capacity of the soil, nitric nitrogen was determined from week to week for the first three inch length of soil from the layer of sulphate of ammonia because total nitrogen did not increase beyond three inch length. With soil containing water equal to 75 per cent. of the water holding capacity nitric nitrogen was determined both for the first three inches and the second three inches of the soil as total nitrogen increased up to six inch length. The actual figures obtained are given below :—

TABLE XV.

Amount in mgrms. of nitric nitrogen per 100 grms. of oven-dry soil.

Time	Soil with water equal to			
	25 per cent. water-holding capacity	50 per cent. water-holding capacity	75 per cent. water-holding capacity	
	0 in.—3in.	0 in.—3 in.	0 in.—3 in.	3 in.—6 in.
1 week	..	..	..	..
2 weeks	3·11	8·52	16·48	21·16
3 weeks	6·42	22·64	19·32	27·42
4 weeks	8·33	28·24	24·02	36·34
5 weeks	9·50	33·18	23·61	43·80
6 weeks	10·37	30·62	22·86	46·72

It seems from these figures that a part of ammoniacal nitrogen is changed, during diffusion, into nitrate nitrogen, the quantity increasing with time and with increasing quantity of water. In the case of soil with water equal to 75 per cent. of the water holding capacity, total nitrogen increased up to six inches. Here the second three inches contain more of nitric nitrogen than the first three inches although total nitrogen is more in the first than in the second three inches. This is perhaps because the nitric nitrogen is pushed more easily than other forms of nitro-

gen. The results about the movement of sulphate of ammonia in soils may be summarised as follows:—

1. In a soil containing such small quantities of moistures as those equal to 25 per cent. of the water holding capacity of the soils sulphate of ammonia diffuses only through a distance of three inches in six weeks.
2. With water equal to 50 per cent. of the water holding capacity the diffusion goes up to six inches but the quantity diffused beyond three inches is small.
3. Even with water equal to 75 per cent. of the water holding capacity the diffusion does not go beyond six inches in six weeks although the quantity diffused is much greater than that with water equal to 50 per cent. of the water holding capacity.
4. The distance through which sulphate of ammonia diffuses is the same for horizontal, vertically downward and vertically upward directions.
5. The quantities diffused are greater in the downward direction and smaller in the upward direction than those along the horizontal direction.
6. With a free surface of soil for evaporation the uppermost part gets dry and the salt which is pulled up stops where capillary connection is broken either due to formation of hard layer or loose dry layer separated from the lower layer.
7. During diffusion a part of the ammoniacal nitrogen is changed into nitric nitrogen, the quantity changed increasing with the increase of water in the soil.
8. Nitrogen in the form of nitrates is more easily diffused than nitrogen in other forms.

VII. Field experiments.

It has been shown by detailed study of the movement of sulphate of ammonia that the salt does not ordinarily move beyond three inches and does not move beyond six inches even if the moisture in the soil is as much as 75 per cent. of the water holding capacity of the soil. The conditions in the laboratory are under control and therefore field experiments were taken up to see whether the laboratory results could be corroborated in the fields. The idea was that if sulphate of ammonia does not move beyond three inches from the place of application it should not be available to the plant when placed beyond that range, and when the salt is placed within three inches it should be within the reach of the plant and its effect should be seen on the plant. For this purpose it was decided to apply sulphate of ammonia at different fixed distances from the plants and observe the effect. A uniform plot of medium black cotton soil usually used for a *rabi* crop was secured on the Poona

Agricultural College Farm to grow *rabi jowar*. The following four treatments were given :—

A. Manure between 0 in. and 3 in. from the plant.
B. Manure between 3 in. and 6 in. from the plant.
C. Manure between 3 in. and 9 in. from the plant.
D. No manure (control).

Each treatment was repeated four times. The treatments and replications were done by randomization. The plan of the plots is shown in Fig. 1.

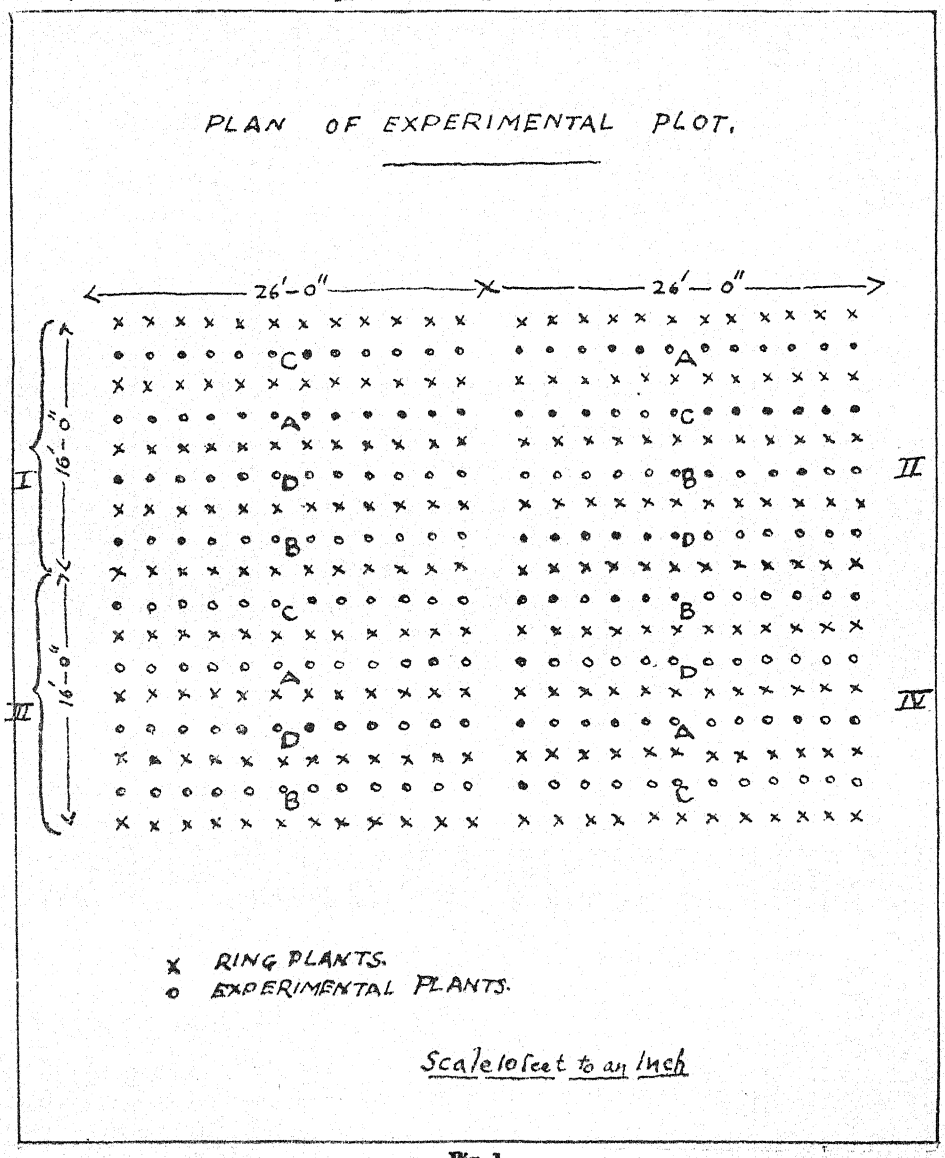


Fig. 1.

The whole experimental plot measured 52 feet by 32 feet. This was divided into 4 blocks of 26 feet by 16 feet. In each block four sub-plots were made measuring 26 feet by 4 feet. Each of these sub-plots received a separate treatment. There were twelve plants under each treatment in a row. The distance between two plants was two feet. The treated plants had rows of untreated plants as rings on both sides. In the beginning 2 to 3 seeds were dibbled on each spot but after germination only one plant was retained.

The plot was given the usual cultivation and was harrowed and lined with a marker. The positions where seed was to be dibbled were marked by fixing small pegs in those positions. Sulphate of ammonia at the rate of 20 lbs. of nitrogen per acre was mixed well with 10 times its weight of soil. The whole mixture was divided into parts equal to the number of plants that were to receive the fertilizer and each plant was given its quota. In the case of treatment "A" the mixture was spread evenly in a ring of three inch diameter round the thin peg where seed was to be dibbled. In treatment "B" the same quantity of mixture was spread between the three inch ring and the six inch ring. Similarly in the case of treatment "C" the same quantity of the fertilizer was spread between the six inch ring and the nine inch ring. In treatment "D" there was no fertilizer given. The fertilizer was put one inch below the surface by digging and refilling the earth.

Sowing was done ten days after the application of the fertilizer and it was all completed in one day. The seed was dibbled two inches deep from the surface. Moisture percentages of the soil from the experimental plot were determined every fortnight. When the crop was quite ripe it was cut as close to the surface of the soil as possible. The following table gives the outturns in pounds :—

TABLE XVI.

Total outturns in pounds.

Block	Treatment			
	A	B	C	D
1	18·50	11·00	9·50	10·25
2	16·50	11·50	10·50	11·50
3	20·50	12·25	9·35	10·25
4	17·00	10·18	10·65	10·50

The following method was used in calculating significance :—

Block	Treatment				Total	Mean
	A	B	C	D		
1	18·50	11·00	9·50	10·25	49·25	12·31
2	16·50	11·50	10·50	11·50	50·00	12·50
3	20·50	12·25	9·35	10·25	52·35	13·08
4	17·00	10·18	10·65	10·50	48·33	12·08
Total	72·50	44·93	40·00	42·50	199·93 (G. T.)	49·97
Mean	18·12	11·23	10·00	10·62	..	12·49 (G. M.)

(1) Sum of squares of 16 figures = 2684·6649

(2) $\frac{\text{Sum of squares of treatment totals}}{4} = \frac{10681{\cdot}2049}{4}$
$= 2670{\cdot}3012$

(3) $\frac{\text{Sum of squares of block totals}}{4} = \frac{10001{\cdot}8739}{4}$
$= 2500{\cdot}4685$

(4) $\frac{\text{Square of the grand total}}{16} = \frac{39972{\cdot}0049}{16}$
$= 2498{\cdot}2503$

Analysis of variance.

Due to	D. F.	Sum of squares	Mean of square
Blocks (3−4)	3	2·2182	0·7394
Treatment (2−4)	3	172·0509	57·3503
Error	9	12·1455	1·3495
Total (1−4) .	15	186·4146	12·42764

$\frac{1}{2}\log_e$
2·02459 $z = 1{\cdot}87454$ exceeds even the one per cent. point according to Fisher's table
0·15005 One per cent. point for $n = 3$ $n^2 = 9$ is ·9724

S. D. $= \sqrt{1{\cdot}3495} = 1{\cdot}161$

The general mean = 12·5

S. D. (per cent.) = 9·29

Standard error for the mean of four plots

$$= \frac{1{\cdot}161}{\sqrt{4}} = 0{\cdot}58$$

Treatments (mean of four figures)

1	2	3	4	Mean	S. E.
18·12	11·23	10·00	10·62	12·50	0·58

Treatment A is significantly superior to all others.

The remaining three treatments do not give statistically significant difference.

The field trials clearly show that a *rabi* crop of *jowar* can use sulphate of ammonia if it is put within three inches from the plants. If it is beyond three inches but within six inches it may be used only to a very small extent but if the fertilizer is beyond six inches from the plants, it cannot be used by the plants at all. These experiments corroborate the results obtained on the movement of sulphate of ammonia.

The moistures in the soil in the *rabi* season were found to be as given below :—

TABLE XVII.

Date of moisture determined	Moisture per cent. on oven-dry basis
2nd September 1931	14·46
17th September 1931	12·40
2nd October 1931	18·8
18th October 1931	16·4
2nd November 1931	15·8
17th November 1931	15·4
2nd December 1931	13·70
7th December 1931	12·48
4th January 1932	11·32
7th January 1932	10·32
2nd February 1932	9·80
18th February 1932	9·24
2nd March 1932	8·46
16th March 1932	8·18

The figure for moisture per cent. in the soil taken on 2nd September 1931 is higher than for moisture in September taken previously, because there was good rain during the period between the 17th of September and the 2nd of October. After this the moisture per cent. went on decreasing throughout. The highest per cent. was 18·8 which works to 36 per cent. of the maximum water-holding capacity of the soil. The moisture varied from 8 to 18 per cent. during the period of the plant growth which means it varied from 15 to 36 per cent. of the maximum capacity of the soil for water. It has been shown that sulphate of ammonia can diffuse beyond three inches only when the moisture in the soil is more than 50 per

cent. of its maximum capacity. In the experimental plot, therefore, there was very little chance for the salt to have moved beyond three inches except perhaps on the days (18th and 19th September) on which there was about 3 inches of rain. The results obtained show that even this rainfall did not help much the sulphate of ammonia to move beyond three inches. These experiments therefore show that during the *rabi* season the moisture in the soil is not sufficient to diffuse sulphate of ammonia beyond three inches.

VIII. Root studies of *jowar* (*Andropogon Sorghum*).

Determinations of soil moistures during the *rabi* season show that it is not sufficient to move sulphate of ammonia much beyond three inches, and naturally therefore if the fertilizer is put beyond that limit, it is not likely to be useful to the crop. But the question whether the roots of the *jowar* plant cannot spread so as to reach the fertilizer remains undecided. If the roots spread out in all directions to about 9 in. to 12 in. the fertilizer would be available. It was therefore decided to study the root system of *jowar* under *rabi* conditions in the Deccan. For this purpose, individual plants in the normal condition of growth were selected and the root system was carefully exposed by digging round and washing by jets of water. The positions of the roots were carefully noted and drawn on a graph paper by taking careful measurements. Thus a completed picture of the root system was obtained. (Figs. 2-5).

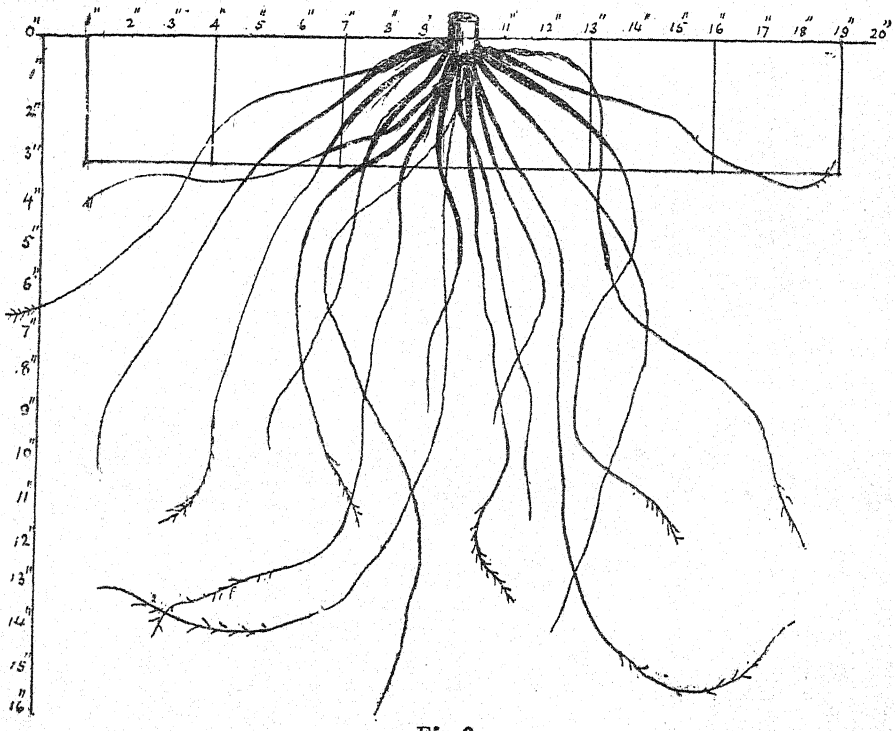


Fig. 2.

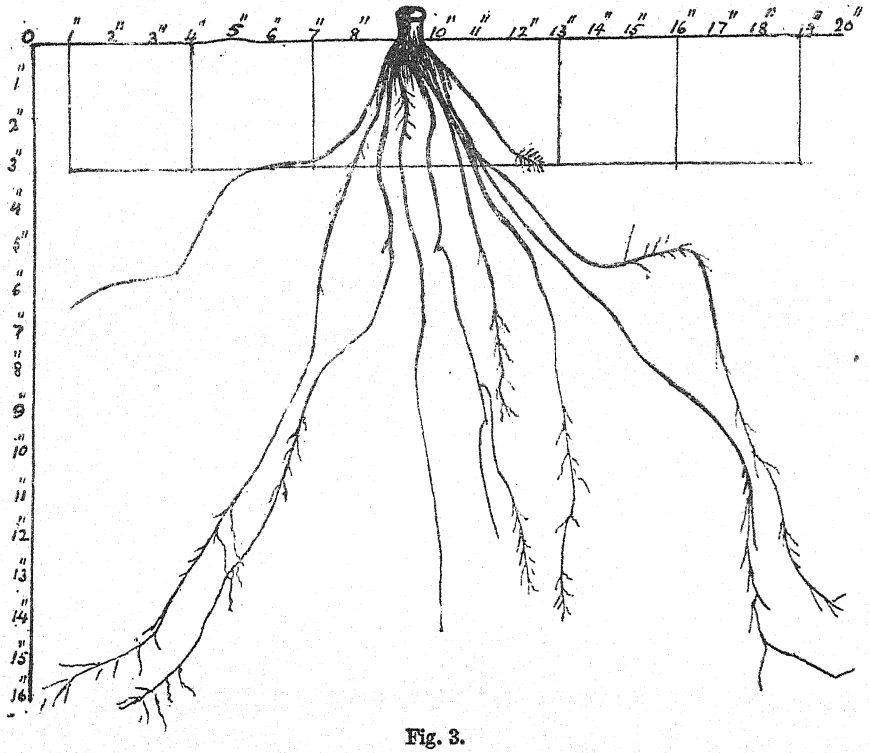


Fig. 3.

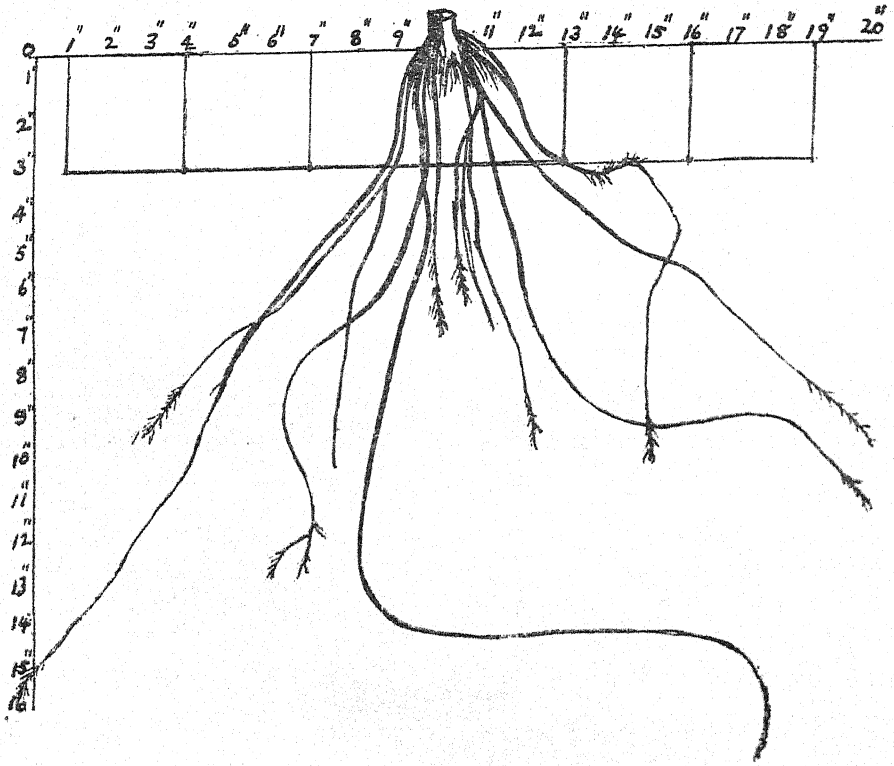


Fig. 4.

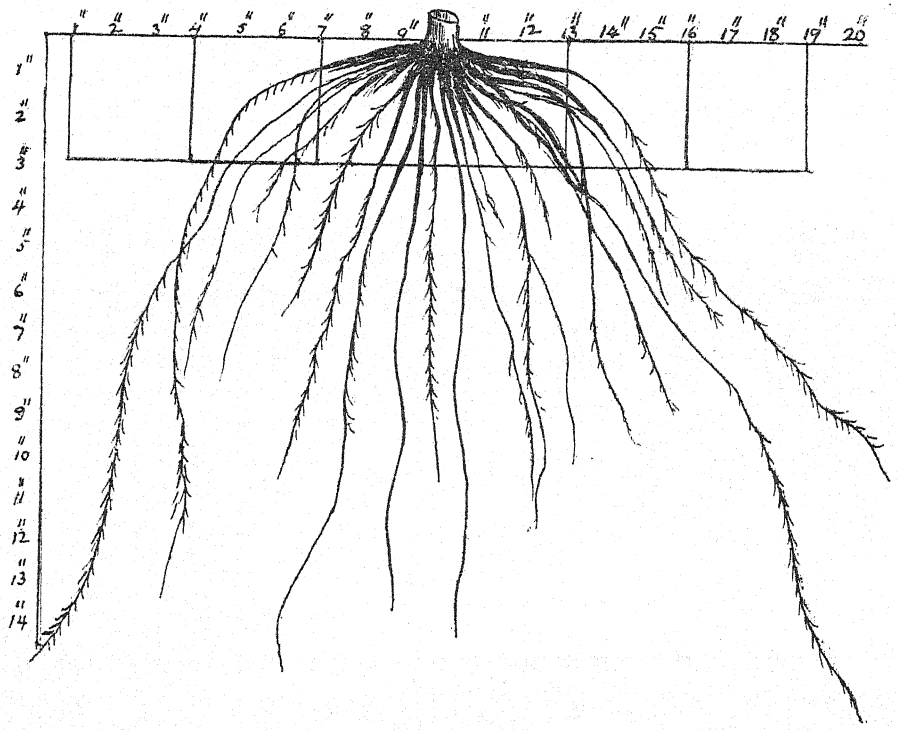


Fig. 5.

The root-system was studied to find out the density of the roots in the various regions from the place where the seed was placed. Three blocks were formed, each measuring three inches in depth from the seed, and horizontally forming rings of three inches, between three and six inches, and between six and nine inches. If the roots fed within three inches, they would utilize the fertilizer in the first block. If they extended into the second ring they would use the fertilizer in the second block and also in the first block because they must pass through the first block. Those roots which extended into the third ring would not only use the fertilizer in the third block but also in the first and the second blocks, as they must pass through the first and the second blocks to reach the third block

The density of the roots was measured in two ways (a) by measuring the total root lengths in each block and (b) by counting the number of root tips that must have passed through each block. As it was not possible to take out and count all the fine rootlets and their feeding portions, it was decided to measure the total length of roots in each block as it would give an indication of the proportion of the feeding mouths of the plant in each block. Portions near the root tips are

chiefly responsible for feeding and the root tips must travel along the directions shown by the roots finally. It was therefore considered necessary to find out how many roots (those that could be traced) passed through each block. This would give an idea as to how many mouths fed during their extension in each block. These two methods would finally indicate the capacity of the plant to feed in each block and make use of the fertilizer there.

The following table gives the comparative figures in root lengths of four plants.

TABLE XVIII.

Total length of roots in inches present in various blocks.

Plant	Block I	Block II	Block III
I.	75	30	6
II.	27	3	*Nil*
III.	28	*Nil*	*Nil*
IV.	38	12	*Nil*

The figures at once indicate that most of the length of the roots lies in the first block. But the percentage figures will be better in giving a comparative idea.

TABLE XIX.

Percentage of root lengths in each block.

Plant	Precentage of total root length in		
	Block No. I	Block No. II	Block No. III
I.	67	26	6
II.	90	10	*Nil*
III.	100	*Nil*	*Nil*
IV.	76	24	*Nil*

The figures clearly show that of the total length of roots in the three blocks the largest proportion lies in the first block and a very small proportion in the second block and very little in the third block. This explains very clearly why a fertilizer placed beyond three inches is not available to the plants.

If the number of root tips that must have fed in the respective blocks are considered the same view is confirmed thus :—

TABLE XX.

Number of root tips that must have fed in the various blocks.

Plant	Block I	Block II	Block III
I.	18	7	2
II.	11	2	0
III.	12	0	0
IV.	23	10	0

This conclusively explains why the *jowar* plant cannot utilise the fertilizer placed beyond three inches from the place of the seed.

IX. CONCLUSIONS.

1. In a soil containing such small quantities of moistures as those equal to 25 per cent. of water holding capacity of the soils, sulphate of ammonia diffuses only through a distance of three inches in six weeks.

2. With water equal to 50 per cent. of the water holding capacity the diffusion goes up to six inches but the quantity diffused beyond three inches is small.

3. Even with water equal to 75 per cent. of the water holding capacity the diffusion does not go beyond six inches in six weeks although the quantity diffused is much greater than that with water equal to 50 per cent. of the water holding capacity.

4. The distance through which sulphate of ammonia diffuses is the same for horizontal, vertically downward and vertically upward directions.

5. The quantities diffused are greater in the downward direction and smaller in the upward direction than those along the horizontal direction.

6. With a free surface of soil for evaporation the uppermost part gets dry and the salt which is pulled up stops where capillary connection is broken either due to formation of hard layer or loose dry layer separated from the lower layer.

7. During diffusion a part of the ammoniacal nitrogen is changed into nitric nitrogen, the quantity changed increasing with the increase of water in the soil.

8. Nitrogen in the form of nitrates is more easily diffused than nitrogen in other forms.

9. The field trials clearly show that a *rabi* crop of *jowar* can use sulphate of ammonia if it is put within three inches from the plants. If it is beyond three inches but within six inches it may be used only to a very small extent but if the fertilizer is beyond six inches from the plants it cannot be used by the plants. These experiments corroborate the results obtained on the movement of sulphate of ammonia.

10. The study of the root system of *jowar* shows that a very large portion of the roots feed within three inches from the plant and any fertilizer to be useful must come within this range.

11. The experiments explain why sulphate of ammonia when put beyond three inches from the plant is not likely to be used by *rabi jowar*.

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A STATISTICAL STUDY OF THE GROWTH OF MAIN STEM IN COTTON.

BY

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INTRODUCTORY.

The growth of field crop has been very widely studied and several mathematical equations have been devised from time to time to interpret the growth curves in a simple and easily comprehensible form. With the advancement of genetics, the phenomenon of hybrid vigour further emphasized the need of some simple but accurate measure for growth. There are two theories in the field to account for the cause of hybrid vigour. One of these assumes the existence of some unexplained physiological stimulation resulting from the union of two unlike gametes, while in the other hypothesis stress is laid on the combined action of the dominant favourable genes coming together from the two parents. It is, however, beyond the scope of the present paper to discuss any of these theories, but an attempt will be made to fit equations to the growth in height of main stem of cotton varieties grown at Lyallpur and thereby illustrating a fruitful line of application of mathematics to biology.

Blackman [1919] formulated his theory of 'Compound Interest Law and Plant Growth' in 1919, and devised an equation for the growth curves. He stated that 'for the highest production of vegetable matter by a single plant, two factors are necessary—large seed and a high economy in working represented by a large

efficiency index'. The efficiency index 'r' as a measure of physiological importance was criticized by Kidd, West and Briggs [1920]. But Blackman [1920] established the importance of 'r' as a measure of average growth rate even if growth did not follow the exponential law. Fisher [1921] in criticizing certain measures of growth rate advocated by Kid, West and Briggs said 'the correct measure for the mean value of the relative growth rate over any period, long or short, is that advocated by Blackman under the name of the 'efficiency index'. This method of expressing growth rate has been very widely adopted and Ashby [1930, 1932] has recently published his results on the genetics of hybrid vigour and the inheritance of 'efficiency index' in maize.

At the conference on Cotton Growing Problems held at the Shirley Institute, Didsbury, Maskell [1930] laid great stress at presenting the developmental data of cotton in some simple form so that comparisons could be easily made between different stations. This, he said, would afford facilities for finding out the effects of change in the intensity of environmental factors upon the development of cotton. Heath [1932] has published the results of fitting an exponential curve to the height measurement data of some varieties of cotton grown at Barberton, South Africa. He fitted curves of the form :—

$$H = Ae^{bT}$$

Where H=height in centimeters, A=a constant (the theoretical height at the time of germination), e=the base of the Naperian logarithms, T=time in days from germination and b=the relative growth rate per day. He has, in his paper, pointed out the analogy of 'b' to Blackman's efficiency index 'r' and has finally suggested that 'if other stations will give the exponential equations for the time curves of height of main stem to the date of first flowering....comparisons of great interest will be possible'. With this point in view, the data of growth in height of the main stem of four varieties of cotton, 4F, 289F, Early Strain and *Mollisoni*, collected at the Cotton Research Farm, Lyallpur, since 1926 has been examined in detail and exponential curves have been fitted.

It is, however, realised that growth in height of the main stem is not a very accurate measure of the 'growth' of the plant. This may vary, as Bailey and Trought [1927] have suggested, with the availability of water to the plant. The accurate measure of growth would, of course, be the daily increase in dry weight. In this connection, however, the following statement made by Reed [1924] would be of interest. "A plant growing in the field is in dynamic equilibrium with its surroundings, otherwise it is dead. If cloudy days or drought or other changes ensue, the activities of the plant take such a course, that the effect of the changed

conditions is minimized and the growth rate generally suffers little change." Inamdar and Singh [1930] have done exhaustive work on this phase of the cotton plant at Benares. They have fitted logarithmic curves of the form $\log w = A \log t\, a$. But it must be mentioned that the most common form of records of growth of cotton at the various stations are the growth in height records and therefore a statistical treatment of such data would be of great interest.

In the present paper, the method employed by Heath [1932] to find out the 'relative growth rates' of different varieties of cotton has been followed and the average relative growth rate per day for the whole period of growth has also been worked out. The method employed has been described rather in detail and the statistical procedure to compare two regression coefficients by the method of pooled variance [Fisher, 1930] has also been described in full.

The data.

The data comprised of the records of daily measurements of height of main stem taken at the Cotton Research Farm, Lyallpur, during 1926-1932. The varieties of cotton used for this purpose were (i) *G. indicum*, var. *mollisoni*, Gammie (ii), 4F (*G. hirsutum* L.), (iii) 289F (*G. hirsutum* L.) and (iv) Early Strain (*G. hirsutum* L). Daily records of the growth in height of 20 selected average plants of each variety beginning from the time when the plants were 5-10 cm. high were taken every day till the time of cessation of growth. The average increase in height per plant per day was calculated for each variety. Knowing also the average height on the 1st day of measurement, it is a simple process to obtain the mean height on successive days by a process of successive addition of the amount of increase to the value already obtained. These form the original data for work in this paper.

Preliminary examination.

The tabulated average heights were plotted on graph paper in which the horizontal rulings are spaced on a logarithmic scale with the actual values indicated in the margin. These graphs are shown in Figs. 1-4. It was found in all cases that such curves ran practically in straight lines up to the time of first flower. At this stage the average height per plant was 40-50 cm. Heath [1932] also found that up to the stage of first flowering the growth in height followed the Compound Interest Law after which the curves were deflected in a rather irregular way. This is to be expected because " profound physiological and chemical changes occur in all higher plants during the period of flowering and seed formation. In the annual plants the seed and associated tissues draw heavily on food reserves and other necessary materials " [Murneek, 1932].

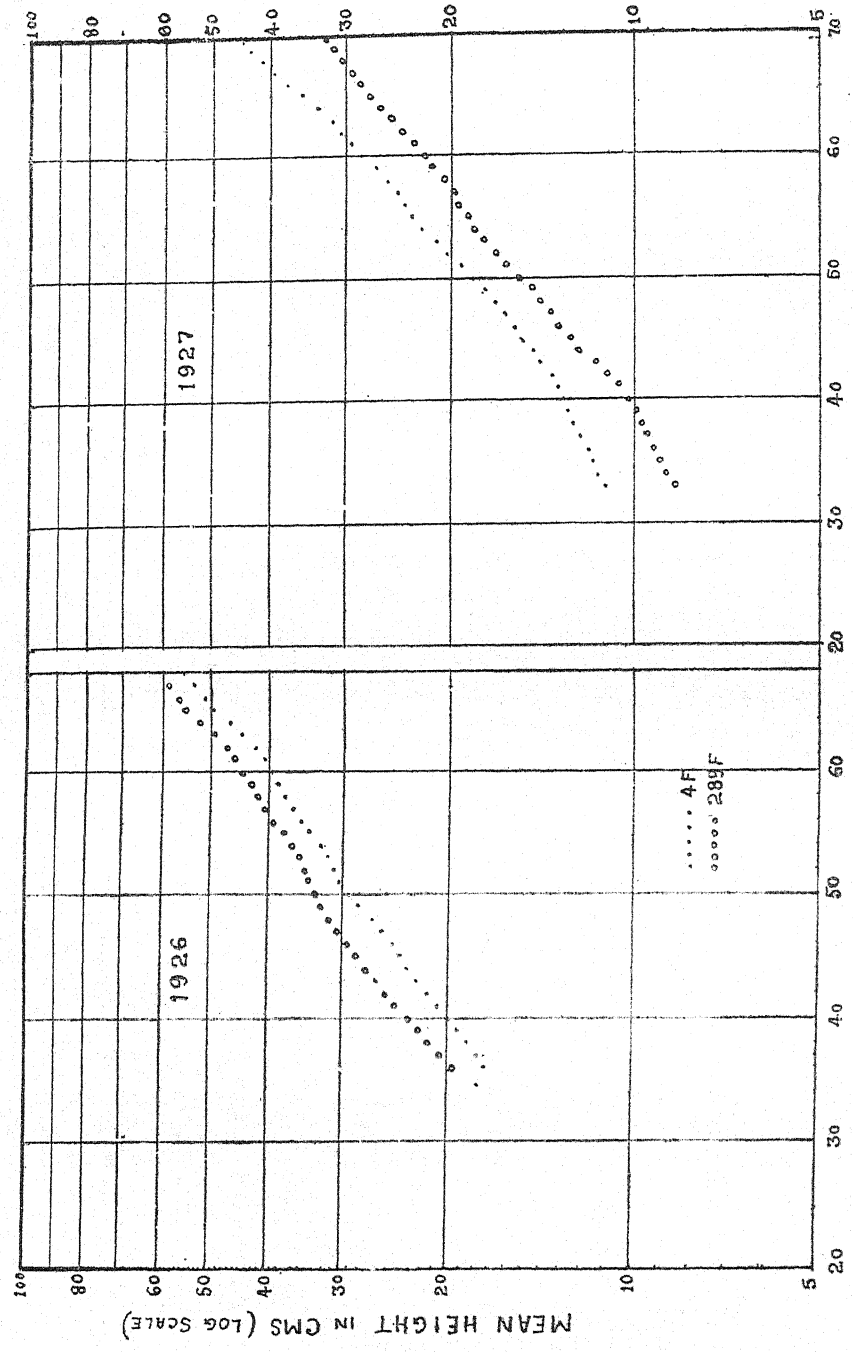

MEAN HEIGHT IN CMS (LOG SCALE)
DAYS FROM GERMINATION
1926
1927
4F
289F

Fig. 1.

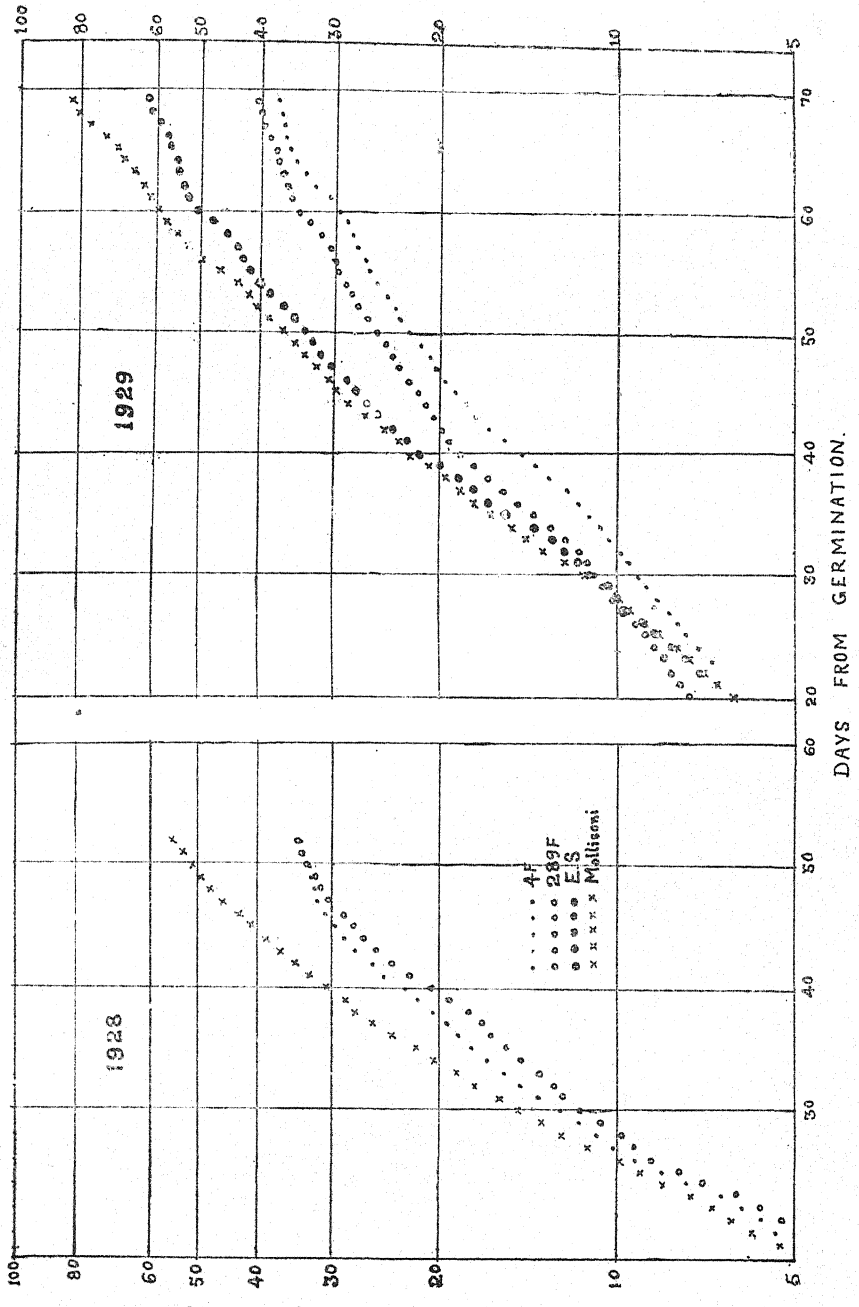

1929
1928
4F
289F
E.S
Malligani
MEAN HEIGHT IN CMS (Log scale)
DAYS FROM GERMINATION.

Fig. 2.

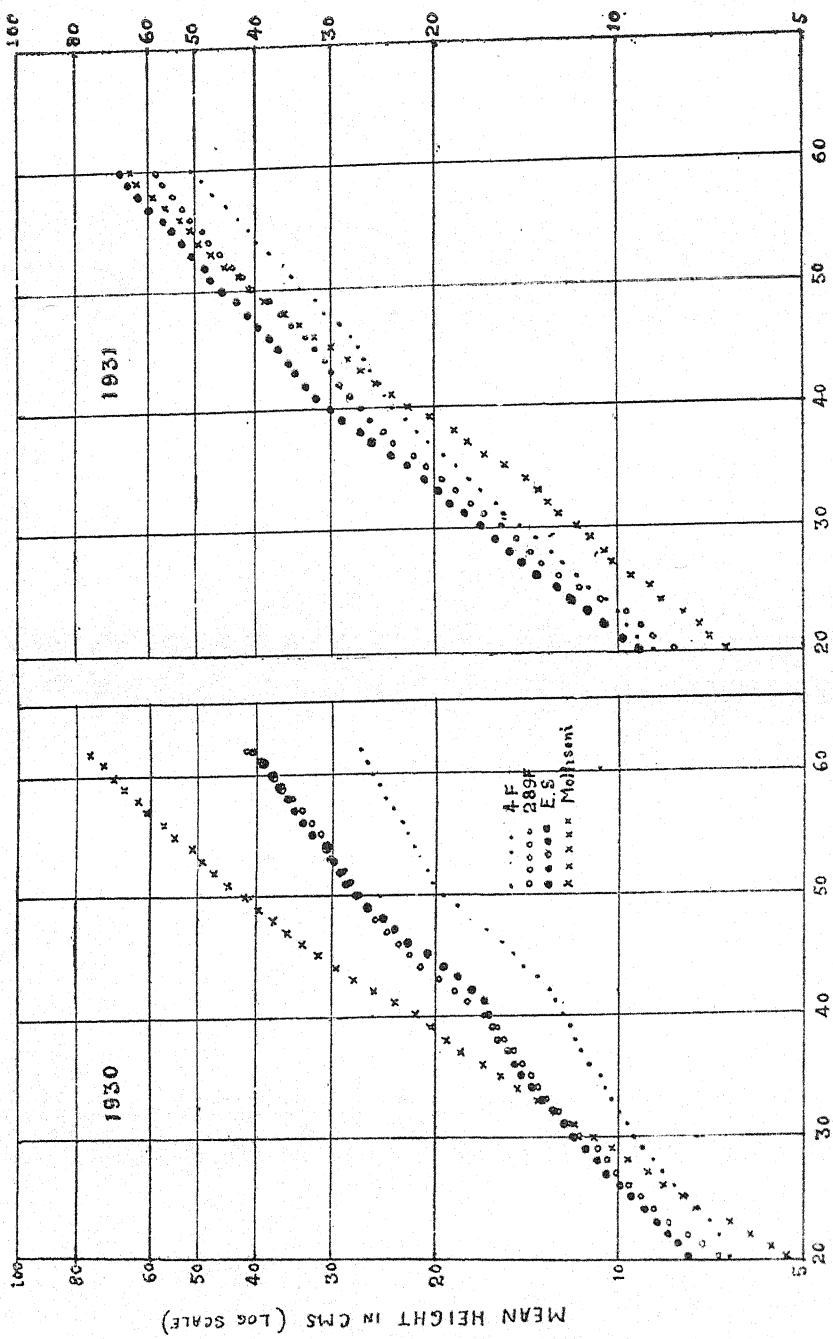

1931
1930
4F
289F
E.S
Mollisani
MEAN HEIGHT IN CMS (LOG SCALE)
DAYS FROM GERMINATION

Fig. 8.

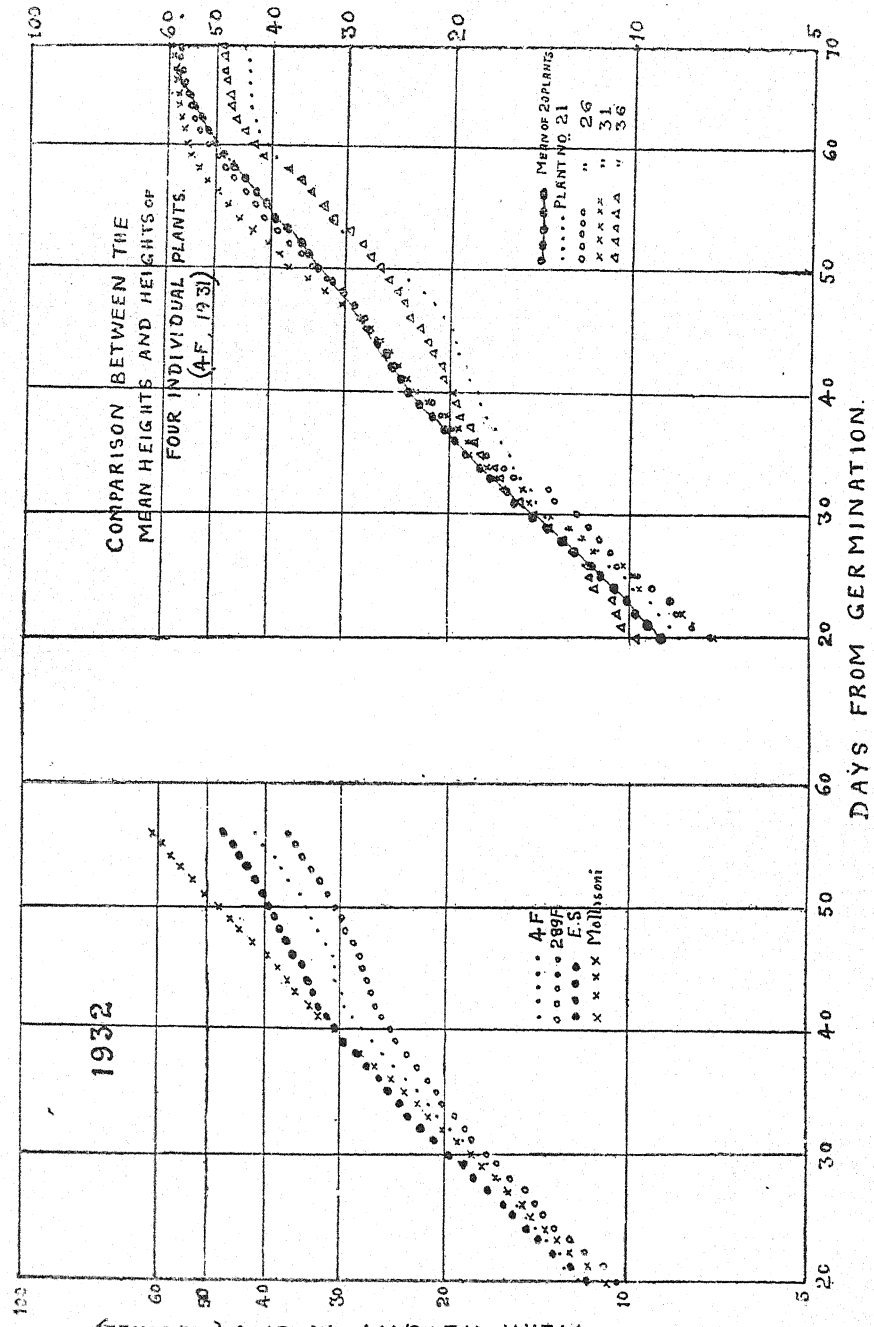

COMPARISON BETWEEN THE MEAN HEIGHTS AND HEIGHTS OF FOUR INDIVIDUAL PLANTS. (4F, 1931)
1932
MEAN OF 20 PLANTS
PLANT NO. 21
" 26
" 31
" 36
4F
299F
E.S.
Mollisoni
MEAN HEIGHT IN CMS (Log Scale)
DAYS FROM GERMINATION.

Fig. 4.

These figures also show that the different curves make different angles with the time axis and as these angles are proportional to the value of 'b' in the equation $H=Ae^{bT}$ it must be inferred that the efficiency index varies a good deal in the different varieties and in different years (Table IV).

It may also be remarked that by working with averages the individual peculiarities of plants as regards their growth are largely smoothed out as may be seen in Fig. 4 by comparing the curves for four particular plants with that of the general average of 20 plants.

Evaluation of the parameters.

By taking common logarithms of both sides of the equation

$$H=Ae^{bT}$$

we get $$\log_{10} H = \log_{10} A + \log_{10} e \times b \times T.$$

Regarding $\log_{10} H$ as the dependent variate (y) and T as the independent variate (x) the problem reduces to fitting the regression equation

$$Y = a + k\,(x - \bar{x}).$$

Table I.

Evaluation of parameters for Mollisoni (*1929*).

No. of days from germination (x)	Mean height cm.	$\text{Log}_{10}H$ (y)	Summation	$(\text{Log}_{10}H)^2$ (y^2)
20	5·38	0·731	33·282	·534361
21	5·77	0·761	32·551	·579121
22	6·08	0·784	31·790	·614656
23	6·38	0·805	31·006	·648025
24	6·74	0·829	30·201	·687241
25	7·11	0·852	29·372	·725904
26	7·56	0·878	28·520	·770884
27	7·98	0·902	27·642	·813604
28	8·49	0·929	26·740	·863041
29	8·98	0·953	25·811	·908209
30	9·54	0·979	24·858	·958441
31	10·00	1·000	23·879	1·000000
32	10·58	1·024	22·879	1·048576

TABLE I—*contd.*

No. of days from germination (x)	Mean height cm.	$\text{Log}_{10}H$ (y)	Summation	$(\text{Log}_{10}H)^2$ (y^2)
33	11·29	1·053	21·855	1·108809
34	12·07	1·082	20·802	1·170724
35	13·31	1·124	19·720	1·263376
36	14·08	1·149	18·596	1·320201
37	14·89	1·173	17·447	1·375929
38	16·15	1·208	16·274	1·459264
39	17·31	1·238	15·066	1·532644
40	18·47	1·266	13·828	1·602756
41	19·59	1·292	12·562	1·669264
42	20·87	1·320	11·270	1·742400
43	22·14	1·345	9·950	1·809025
44	23·33	1·368	8·605	1·871424
45	24·71	1·393	7·237	1·940449
46	26·44	1·422	5·844	2·022084
47	28·21	1·450	4·422	2·102500
48	29·74	1·473	2·972	2·169729
49	31·55	1·499	1·499	2·247001
	Total	33·282	576·480	35·559642

By calculating the two parameters

$$a = \bar{y}$$

and

$$k = \frac{S\ y\ (x - \bar{x})}{S\ (x - \bar{x})^2}$$ [Fisher, 1930]

The values of A and b can then be easily derived by the relations

$$b = k/\log_{10} e$$

and

$$\log_{10} A = a - k\bar{x}_4$$

($\bar{x}$ and $\bar{y}$ are the means estimated from the sample).

The working is shown in Table I for *Mollisoni*. The columns are practically self explanatory and the sums are given at the feet of the columns.

The sum of column 3 divided by the total number n' (30 in this case) gives the value of $\bar{y}=a=$ 1·1094.

To calculate $Sy\ (x-\bar{x})$ the sum of the figures in the next column is taken. This column is formed by a process of successive addition starting from the bottom. From the sum of this column is subtracted $\frac{n'+1}{2}$ times the sum of the previous column to get the value of the 'sum of products', thus:—

Sum of column 4	=	576·480
15·5 × sum of column 3	=	515·871
Difference	=	60·609

To evaluate $S\ (x-\bar{x})^2$ in this case is very simple as we have only to find $\frac{1}{12}\ n'\ (n'^2-1)$.

This gives $\frac{1}{12} \times 30 \times (900-1) = 2247{\cdot}5$

The value of $k = \frac{S\ y\ (x-\bar{x})}{S\ (x-\bar{x})^2} = \frac{60{\cdot}609}{2247{\cdot}5} = 0{\cdot}0269$

Hence 'b' $= \frac{0{\cdot}0269}{{\cdot}4343} = {\cdot}0620$

Also the value of 'a' = 1·1094.

$\bar{x}$ is found to be 34·5 from column 1.

Hence $\log_{10} A = 1{\cdot}1094 - 0{\cdot}0269 \times 34{\cdot}5 = 0{\cdot}1779$.

From tables of logarithms, therefore $A = 1{\cdot}51$.

Thus in this case the equation works out to be

$$H = 1{\cdot}51e^{{\cdot}0620T}$$

To calculate the Standard Error of 'b'.

The last column of Table I gives the values of the squares of numbers in column 3. The sum of this column gives $S\ (y^2)=38{\cdot}559642$.

Subtracting from this $\frac{[S\ (y)]^2}{n'}$ or $\frac{(33{\cdot}282)^2}{30}$

We get $S\ (y-\bar{y})^2 = 1{\cdot}63659$

This is the total sum of squares of deviations.

To find the residual sum of squares, *viz.*, $S\ (y-Y)^2$, we have to subtract from this quantity the product of k^2 and $S\ (x-\bar{x})^2$.

This gives $S\ (y-Y)^2 = 28\ s^2 = {\cdot}01028$ (See below).

The remaining steps to calculate the Standard Error of 'k' are shown below :—

$$S\,(x-\bar{x})^2 = \frac{n/\,(n'^2-1)}{12}$$

$$= 2247{\cdot}5$$

$$k = {\cdot}0269$$

$$S\,(y-\bar{y})^2 = 1{\cdot}63659$$

$$k^2\,S\,(x-\bar{x})^2 = 1{\cdot}62631$$

$$S\,(y-Y)^2 = {\cdot}01028$$

$$s^2 = {\cdot}00037$$

$$\frac{s^2}{S\,(x-\bar{x})^2} = ({\cdot}00040)^2$$

Standard Error of $k = \pm\,{\cdot}00040$

$$t = \frac{{\cdot}0269}{{\cdot}00040} \text{ is } > 67$$

No. of degrees of freedom $(n) = 28$.

Also the Standard Error of 'b' $= \dfrac{\text{S. E. of } k}{{\cdot}4343} = \pm{\cdot}00092.$

Applying Fisher's 't'-test, the value of 't' is found to be very large and therefore statistical significance of 'k' is established.

In a similar manner the parameters A and 'b' and the Standard Errors of 'b' in other cases are worked out and are tabulated in Table II.

TABLE II.

Exponential height curves. (*Daily observations.*)

Season	Variety	Number of observations 'n'	Equation $H = Ae^{bT}$	Relative growth rate = 'b'	Standard Error of 'b'
1926-27 . .	4 F . .	30	$H = 5{\cdot}01e^{{\cdot}0355T}$.	·0355	·00040
	289 F . .	30	$H = 6{\cdot}22e^{{\cdot}0331T}$.	·0331	·00107
1927-28 . .	4 F . .	30	$H = 3{\cdot}07e^{{\cdot}0368T}$.	·0368	·00070
	289 F . .	30	$H = 2{\cdot}40e^{{\cdot}0370T}$.	·0370	·00054

TABLE II—*contd.*

Season	Variety	Number of obversation 'n'	Equation $H = Ae^{bT}$	Relative growth rate = 'b'	Standard Error of 'b'
1928-29 . .	4 F . .	30	$H = 1{\cdot}480e^{\cdot0632T}$.	·0682	·00159
	289 F . .	30	$H = 1{\cdot}30e^{\cdot0694T}$.	·0694	·00121
	Mollisoni . .	30	$H = 1{\cdot}58e^{\cdot0739T}$.	·0739	·00121
1929-30 . .	4 F . .	25	$H = 2{\cdot}62e^{\cdot0463T}$.	·0463	·00095
	289 F . .	30	$H = 3{\cdot}08e^{\cdot0433T}$.	·0433	·00086
	Early Strain .	25	$H = 1{\cdot}92e^{\cdot0596T}$.	·0596	·00120
	Mollisoni . .	30	$H = 1{\cdot}51e^{\cdot062T}$.	·0620	·00092
1930-31 . .	4 F . .	30	$H = 3{\cdot}23e^{\cdot0357T}$.	·0357	·00084
	289 F . .	30	$H = 3{\cdot}05e^{\cdot0423T}$.	·0423	·00055
	Early Strain .	30	$H = 3{\cdot}39e^{\cdot0407T}$.	·0407	·00071
	Mollisoni . .	30	$H = 1{\cdot}54e^{\cdot0663T}$.	·0663	·00044
1931-32 . .	4 F . .	25	$H = 3{\cdot}40e^{\cdot0479T}$.	·0479	·00058
	289 F . .	30	$H = 3{\cdot}12e^{\cdot0525T}$.	·0525	·00103
	Early Strain .	25	$H = 2{\cdot}49e^{\cdot0562T}$.	·0562	·00019
	Mollisoni . .	30	$H = 1{\cdot}93e^{\cdot0608T}$.	·0608	·00086
1932-33 . .	4 F . .	25	$H = 5{\cdot}01e^{\cdot0415T}$.	·0415	·00058
	289 F . .	25	$H = 4{\cdot}67e^{\cdot0423T}$.	·0423	·00087
	Early Strain .	25	$H = 4{\cdot}97e^{\cdot0454T}$.	·0454	·00118
	Mollisoni . .	25	$H = 3{\cdot}94e^{\cdot0514T}$.	·0514	·00079

Both the values of 'A' and of 'b' show considerable variation from variety to variety and from season to season. A large value of A indicates that very rapid growth took place in the initial stages and *vice versa* [Heath, 1932].

The values of the efficiency indices are plotted graphically in Fig. 5. These curves show that the Desi variety *Mollisoni* has the highest relative growth rate or efficiency index in all the years and that Early Strain follows it fairly closely. Between 4 F and 289 F there is not any appreciable difference. It is interesting to compare the values of the relative growth rates thus found for the cottons grown in the Punjab with Heath's figures for the South African varieties grown at Barberton. The figures given by Heath are reproduced in Table III.

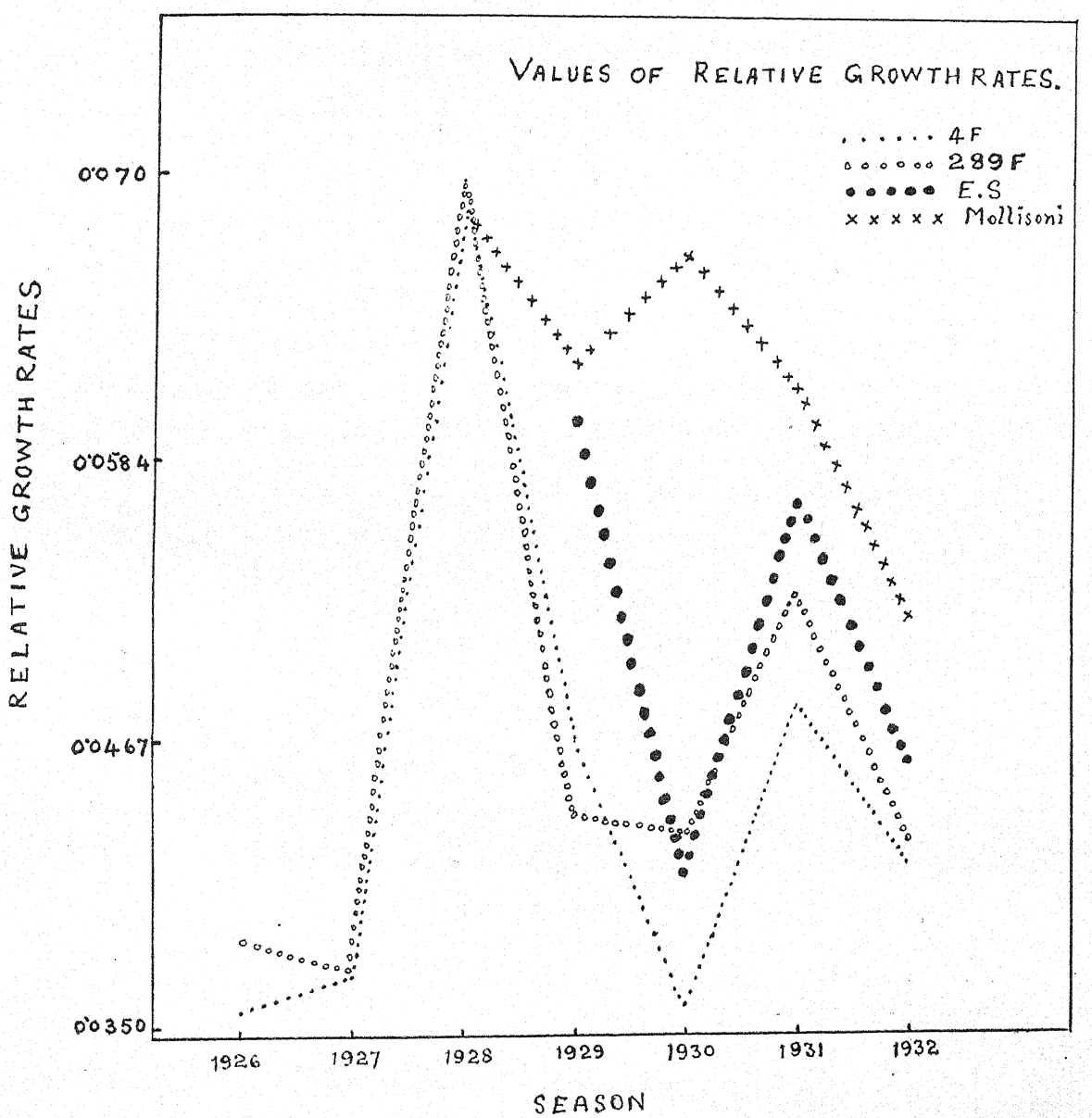


Fig. 5.

TABLE III.

Exponential height curves up to time of first flowers (*Figures given by Heath*).

(2-daily observations.)

Season	Variety	No. of observations 'n'	Equation $H=Ae^{bT}$	Relative growth rate 'b'	Standard deviation of 'b'
1928-29 . .	Z. 1/9	23	$H=5{\cdot}039e^{{\cdot}0320T}$	·0320	·00028
1929-30 . .	U. 4/2 (M. W.)*	18	$H=3{\cdot}850e^{{\cdot}0355T}$	·0355	·00034
1929-30 . .	U. 4/2 (M. S.)†	18	$H=4{\cdot}197e^{{\cdot}0331T}$	·0331	·00071
1930-31 . .	U. 4/2·920 SB	21	$H=4{\cdot}896e^{{\cdot}0419T}$	·0419	·00217

* Mealies' winter treatment.

† Mealies' spring treatment.

The values of A and 'b' and of the Standard Errors of 'b' compare favourably with those obtained at Lyallpur although the range of variation in A is larger in the case of Indian Cottons. This is but to be expected, considering the larger number of varieties and a period covering seven years.

COMPARISON OF RELATIVE GROWTH RATES.

In order to compare two regression coefficients the same procedure is adopted as in the case of comparison of two mean values. The residual sum of squares in the two cases are added and divided by the total number of degrees of freedom. This gives the value of the average variance s^2.

s^2 divided by $S\,(x—\bar{x})^2$ in each case and added gives the variance of the difference $k—k'$. In this case as the number n' is the same *viz.*, 30 in both cases, the value of $S\,(x—\bar{x})^2$ is also the same (=2247·5). Hence the working of the Standard Error of $k—k'$ is fairly simple as shown below.

$S\,(y—Y)^2$ in the case of *Mollisoni* (1929). . . = ·01028

$S\,(y—Y)^2$,, 289 F (1929) . . . = ·00887

Adding these two, we have $n\,s^2$ $(n = 56)$ . . = ·01915

Therefore s^2 . = ·0003419

$$\text{and } \frac{2\,s^2}{2247\cdot5} = \frac{\cdot0006838}{2247\cdot5}$$

$$\text{Standard Error of } k—k' \quad . \quad . \quad . = \sqrt{\frac{\cdot0006838}{2247\cdot5}}$$

$$= \pm \cdot000552$$

$$\text{Also } k—k' \quad . \quad . = \cdot0269 - \cdot0188 = \cdot0081$$

$$\text{Hence } t = \frac{\cdot0081}{\cdot000552} > 14$$

and $n = 56$

The difference is thus highly significant. Similarly comparisons between relative growth rates are made in the case of other varieties also and the summary of results is shown in Table IV.

Fisher's 't'-test shows that in the case of most of the pairs compared the difference in relative growth rates or efficiency indices during this very important stage of the plant's life is statistically significant. Between 4 F and 289 F alone there is no statistical significance as can be seen from Table IV except in 1931-32. The table also shows that although Early Strain approaches the Desi variety *Mollisoni* nearest, except in 1929-30, there is still a significant difference between the efficiency of the American and Desi varieties.

TABLE IV.

Showing the comparison of relative growth rates of different varieties in different seasons.

Season	Varieties compared		Difference in relative growth rate (1—2)	Value of 't'	One per cent. value of 't'	Remarks
	1	2				
1926-27 . .	289 F	4 F	—0·0024	2·088	<2·750 >2·576	Not significant
1927-28 . .	289 F	4 F	0·0002	0·2260	do.	Not significant
1928-29 . .	289 F	4 F	0·0012	·5992		Not significant
	Mollisoni	4 F	·0057	2·845	do.	Significant
	Mollisoni	289 F	0·0045	2·628		do.
1929-30 . .	289 F	4 F	—0·0030	2·259		Not significant
	Early Strain	4 F	0·0133	8·50		Significant
	Mollisoni	4 F	0·0157	11·78	do.	do.
	Early Strain	289 F	0·0163	11·04		do.
	Mollisoni	289 F	0·0187	14·67		do.
	Mollisoni	Early Strain	·0024	1·620		Not significant
1930-31 . .	289 F	4 F	·0066	6·592		Significant
	Early Strain	4 F	·0050	4·563		do.
	Mollisoni	4 F	·0306	32·370	do.	do.
	Early Strain	289 F	—·0016	1·782		Not significant
	Mollisoni	289 F	·0237	33·53		Significant
	Mollisoni	Early Strain	·0256	30·65		do.
1931-32 . .	289 F	4 F	·0046	3·389		Significant
	Early Strain	4 F	·0083	13·600		do.
	Mollisoni	4 F	·0129	11·150	do.	do.
	Early Strain	289 F	·0037	2·900		do.

TABLE IV—*contd.*

Season	Varieties compared		Difference in relative growth rate (1—2)	Value of 't'	One per cent. value of 't'	Remarks
	1	2				
1931-32 . .	*Mollisoni* .	289 F	·0083	6·181	<2·750 >2·576	Significant
	Mollisoni .	Early Strain	·0046	4·341	do.	do.
1932-33 . .	289 F .	4 F	·0008	0·7570		Not significant
	Early Strain	4 F	·0039	2·944		Significant
	Mollisoni .	4 F	·0099	9·679		do.
	Early Strain	289 F	·0031	2·108		Probably significant
	Mollisoni .	289 F	·0041	3·486	do.	Significant
	Mollisoni .	Early Strain	0060	4·221		do.

Finally the average relative growth rates and final heights and initial heights are given in the following table :—

TABLE V.

Average relative growth rates, final and initial heights.

Season	Variety	First measurement in cm. $=H_1$	Final height in cm. $=H_2$	No. of days H_1 to H_2 $=n'$	Average relative growth rate per day $\frac{\log_e H_2 - \log_e H_1}{n'}$
1926-27 . . .	4 F . .	17·00	109·80	92	·0203
	289 F . .	19·89	126·36	92	·0201
1927-28 . . .	4 F . .	11·00	115·83	102	·0231
	289 F . .	8·36	117·21	102	·0258
1928-29 . . .	4 F . .	4·55	78·17	112	·0254
	289 F . .	3·81	125·14	112	·0312
	Mollisoni .	5·31	141·32	112	·0293

TABLE V—*contd.*

Season	Variety	First measurement in cm. $=H_1$	Final height in cm. $=H_2$	No. of days H_1 to H_2 $=n'$	Average relative growth rate per day $\frac{\log_e H_2 - \log_e H_1}{n'}$
1929-30	4 F	6·98	79·02	109	·0223
	289 F	7·55	84·94	109	·0222
	Early Strain	6·17	122·46	109	·0274
	Mollisoni	5·38	146·14	109	·0317
1930-31	4 F	5·42	76·40	120	·0205
	289 F	4·88	94·74	121	·0245
	Early Strain	6·54	81·03	105	·0239
	Mollisoni	3·40	177·31	106	·0372
1931-32	4 F	8·85	88·73	102	·0226
	289 F	8·08	114·66	110	·0241
	Early Strain	9·29	125·18	102	·0255
	Mollisoni	6·67	159·87	110	·0289
1932-33	4 F	11·65	121·66	106	·0221
	289 F	9·90	133·33	106	·0245
	Early Strain	11·10	140·00	106	·0239
	Mollisoni	10·43	187·13	106	·0272

As an average measure of relative growth rates for the whole period of growth the above values are valuable. In order to study how far these values compare with the corresponding values of '*b*' the coefficient of correlation was worked out taking all the 23 pairs of values available thus disregarding the effect of varieties and of seasons.

A high correlation of $r=0{\cdot}7640$ ($P<{\cdot}01$) was obtained. This shows that as a general index of the relative growth rate for the whole period of growth a knowledge of the value of '*b*' found from height measurements extending only over a period of about a month is of very great value.

Summary.

1. Exponential curves of the form $H=Ae^{bT}$ have been fitted to height measurement data of cotton covering a period of seven years and a comparison of the values of 'b' or the 'efficiency index' made. American varieties have numerically less relative growth rate than the Desi variety *Mollisoni*.

2. The values of 'b' estimated for Punjab Cottons compare favourably with those found by Heath for South African varieties grown at Barberton.

3. Full statistical details are given for fitting the curve and for comparison of two values of 'b' by the method of pooled variances.

4. There is a high coefficient of correlation between the value of 'b' and the average relative growth rate for the whole period of growth.

Acknowledgments.

We have great pleasure in acknowledging that the data of the growth in height of the different varieties of cotton dealt with were collected under the guidance of Mr. T. Trought during 1926-30.

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THE AZOTOBACTER PLAQUE TEST OF SOIL DEFICIENCY AS APPLIED TO SOME INDIAN SOILS.

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(With Plate XIII)

A knowledge of the fertilizer requirements of a soil is of primary importance to the scientific agriculturist and the practical farmer. It is recognized that some fields are more deficient in fertility than others situated in the same area, the fertility of a field being dependent upon the way it has been handled and cropped previously, and for this reason it is not possible to judge the mineral deficiencies of the soils in all the fields by resorting to an analysis of a single typical sample from the whole area. Samples of soil from each field must be analysed separately. Many chemical methods have been suggested for the determination of the available plant food but they are likely to prove laborious and expensive when it is intended to handle expeditiously samples of soil from several plots of land. The limited value of the chemical methods for this purpose has served to stimulate research in biological methods.

It is well known that the microbiological activity in the soil depends on the presence of sufficient nutrient materials and favourable environmental conditions and that the organisms belonging to the azotobacter group are particularly sensitive to soil acidity and lack of phosphate. Taking advantage of this fact Christensen [1907] devised a method of inoculating azotobacter into mannite solution with and without calcium carbonate, to which is added the soil whose lime requirement is to be tested ; whilst Niklewski [1912] claimed that the available phosphate in a soil could approximately be determined by introducing a given amount of soil in mannite solution free from phosphorus, inoculating with a culture of azotobacter and noting the scum and the amount of nitrogen fixed after a definite period of incubation. Winogradsky [1926] criticised these methods on the ground that

the organisms were allowed to grow under semi-anærobic conditions, as opposed to the natural habitat; and ultimately a method was developed by him called "la methode des plaques moulees" or the kneaded plate method for directly examining the azotobacter cells on the surface of the soil itself. In his later investigations in collaboration with Ziemiecka [1928] he observed a close correlation between the limiting factors for azotobacter and those for growing plants, and stated, "It is clear that the reaction of these microbes so sensitive to limiting mineral factors can serve to indicate these latter (limiting mineral factors) in the soil and that with a sensitiveness very superior to chemical methods". This method has since been tested by various investigators as it is comparatively simple and is claimed to give information about the fertilizer requirements of soils in a short time. Güittonneau [1929] obtained with French soils excellent correlation between field conditions and the azotobacter growth on soil plaques. Ziemiecka [1929, 1932] who applied it to a study of Polish and Rothamsted soils could also get a correct indication of phosphate supply or deficiency in soils receiving little or no nitrogen manures. Jones [1932] in Canada examined Ontario soils and reported a reasonably satisfactory correlation of the results of the azotobacter soil plaque test and crop returns.

With Winogradsky's work as the basis, Sackett and Stewart [1931] modified the method for finding out mineral deficiencies of Colorado soils and recommended its extensive adoption by others as a routine procedure for determining phosphorus, potash and lime requirements of soils. Recently, Hans Keller [1932] tested it in the case of certain German soils with satisfactory results.

Greene [1932] in Arizona, however, could find correlation with field observations in only 12 per cent. of Arizona soils examined, which are calcareous in nature, and Young [1933] finds that the method is of little value for determining the phosphate deficiencies of Iowa soils. Pittman and Burnham [1932] have not found this test reliable in Utah soils.

In view of the contradictory results obtained by different investigators with various soils, it was decided to apply the test to a few Indian soils in order to study the possibility of adopting it as a routine method.

Technique.

The principle of the method consists in observing the development of the azotobacter colonies on the surface of the soil itself, which has been properly moistened and kneaded and to which the requisite energy in the form of starch has been added. In our experiments we employed Sackett's technique with slight modification.

One hundred grms. of air-dried, sifted soil were mixed with 5·0 grms. of starch. The mixture was divided into four equal parts. No. 1, without any further addition, remained as control; to No. 2 was added 2·5 c. c. of a 3 per cent. solution of potassium sulphate to test for potash deficiency; No. 3 received 2·5 c. c. of a 6 per cent. solution of Na_2HPO_4 to test for phosphate deficiency, while to the fourth was added 2·5 c. c. of a 3 per cent. solution of K_2HPO_4 to test both potash and phosphate deficiencies.

Enough distilled water is added by means of a graduated pipette to No. 1 to give it the consistency of modelling clay and kneaded well. The same amount of water, less the quantity used in nutrient solutions, is added to the other portions also. On a moistened glass plate, this kneaded ball of soil is placed and pressed by the palm of hand to form a circular cake about 1 inch in diameter and the surface smoothened by a wet spatula. The cakes are transferred to a petri dish, which is kept in a moist chamber, to prevent them from drying and incubated at 30°C. for 3 days.

Provided the conditions are favourable, moist, glistening colonies of azotobacter make their appearance on the surface of the cakes, visible to the naked eye, or better still under a magnifying lens (Plate XIII). Depending upon the vigour of the azotobacter growth, the colonies may assume a dark pigmentation. The interpretation of results depends upon the comparison of growth on the four plaques. If a vigorous growth of azotobacter occurs on all the cakes, the inference is that the soil contains the requisite amount of potash and phosphoric acid and requires no manuring. If the control cake shows no colonies, it means that the soil is deficient in one or more mineral elements. If, on the other hand, growth occurs in one or more cakes to which some particular mineral constituent has been added, the soil is supposed to be deficient in that particular factor, *e.g.*, if growth occurs only in potassium sulphate and K_2HPO_4 cakes, the soil is deficient in potash, if in K_2HPO_4 and Na_2HPO_4 cakes, phosphoric acid is lacking and if growth occurs only in the plaque treated with K_2HPO_4 the soil would respond to both potash and phosphoric acid.

When acid soils having a pH index of less than 6·8 are to be examined, the acidity will have to be neutralized by mixing with a sufficient amount of lime, say 10 per cent. In some cases, where the soil is lacking in the flora, we must have recourse to inoculation with a culture of azotobacter. There can be no objection to this inoculation, because the object of the plaque method is to test whether the soil is suitable for the growth of azotobacter and not whether the organisms are actually present in the soil. Further, the physical condition of the soil is an important consideration for the appearance of these microbes. Very sandy soils are improved by the addition of kaolin, whilst in the case of heavy clays, 50 per cent. quartz sand should be added to improve the texture.

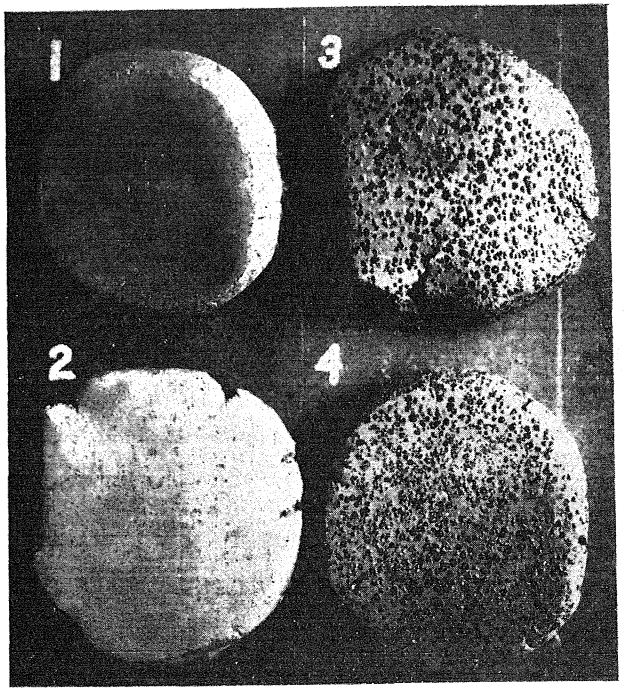


Fig. 1.—Sialkot soil+5 per cent. soil. (*1*) Control. (*2*) 2·5c.c. K_2SO_4. (*3*) 2·5c.c. Na_2HPO_4. (*4*) 2·5c.c. K_2HPO_4.

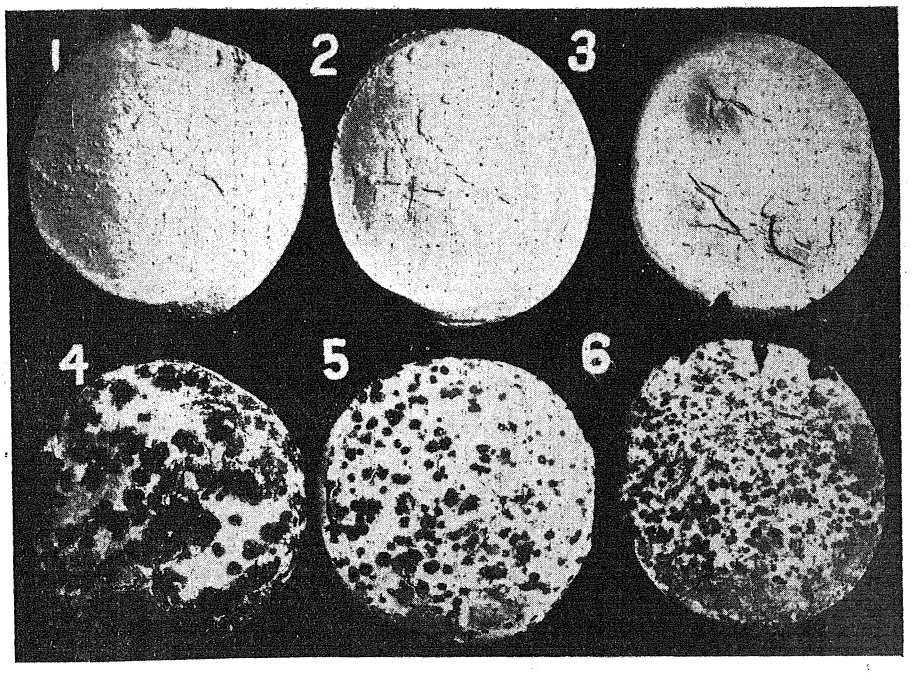
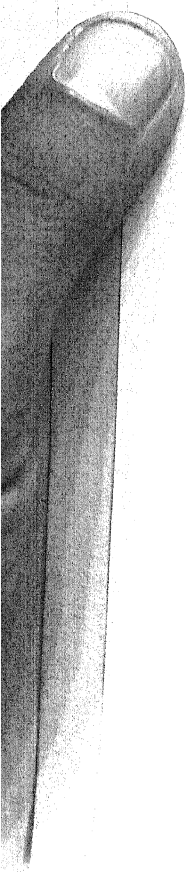


Fig. 2.—Chemical Section Pot Culture House plots. Top row—control. Lower row—2·5c.c. K_2HPO_4. (*1*), (*4*) Nitrogen. (*2*), (*5*) Potash. (*3*), (*6*) Phosphoric acid.



Experimental.

Eight Indian soils of varying pH and physical texture were subjected to this test to find out whether any definite indication could be obtained about their deficiencies in potash and phosphoric acid. The results are shown in the following table :—

Soils	pH	Control	With the addition of		
			K_2SO_4	Na_2HPO_4	K_2HPO_4
Gujranwala	8·1	0	0	+++P	+++
Pusa	7·9	0	0	+	+++P
Kalyanpur	7·3	0	0	++	++++P
Sialkot	7·3	0F	0F	++++P	++++P
Krishnagar	7·3	+F	0	++	++++P
Chinsurah	7·2	0F	0	+++P	+++P
Ranchi.	6·0	0	0	0	0
Dacca	5·9	0	0	0A	0A

0=No azotobacter.
+=Scanty growth.
++=Average growth.
+++ Moderate growth.
++++=Luxuriant dense growth.
P=Pigmented.
A=Actinomycetes.
F=Fungus.

From the above table it will be seen that only one, out of the eight soils tried, gave a few colonies of azotobacter in the control untreated plaque. The addition of potassium sulphate does not improve matters in any case ; whereas response to K_2HPO_4 is clearly indicated in all soils tested, except Dacca and Ranchi which are acidic in character. The effect of Na_2HPO_4 is less marked than that of potassium phosphate, although none of the soils responded to potash when potassium sulphate was added. Probably the potassium phosphate being a better buffer salt did not change the reaction of the soil adversely for the growth of the azotobacter as the sodium salt might have done. We had not adjusted the reaction of the sodium phosphate to pH 7·0 by taking a mixture of Na_2HPO_4 and NaH_2PO_4.

The conclusion to be drawn from these tests according to the originators of the method is that all the soils examined are lacking in available phosphate and will respond to phosphate manuring. We must not, however, lose sight of a possibility

that the test might not be sensitive enough to distinguish between the varying degrees of phosphate requirements of soils.

Consequently, further trials were carried on with soils taken from the permanent manurial plots in the Punjab Field Experimental Area at Pusa: these plots have been receiving the same manurial treatment continuously for 20 years or more and so soils taken from the plots manured with superphosphate should show good growth of azotobacter. In this experiment we compared the control plaques with the K_2HPO_4-treated plaques only. The results appear in the table below :—

—	Farmyard manure	Green manure	Complete minerals	Super-phosphate	Potassium sulphate	Ammon. sulphate +super	Green manure +super	No manure
Control .	+	0	+	++	0	0	0	0
K_2HPO_4 added	++++P	++++P	++++P	++++P	++++P	++++P	++++P	++++

Here again a more vigorous growth of azotobacter with the addition of K_2HPO_4 than in the control cakes is seen in all the samples, including even that from the plot manured with superphosphate for over 20 years, showing that the addition of this fertilizer does not seem to leave the phosphate in the soil readily available for azotobacter.

Since the soil sample from the superphosphate-treated plot was the only one which gave average growth in the control cake, this was tested again, employing the usual mineral solutions. The results appear below :—

Control	++
K_2SO_4	+++
Na_2HPO_4	++ P
K_2HPO_4	++++ P

The above was the only instance in which addition of potassium sulphate was found to have induced azotobacter growth to a greater extent than in the control.

Since sodium phosphate did not encourage the growth of azotobacter to the same extent as potassium phosphate the conclusion according to the originators of the technique would be that the soil needs potash. But it is also possible to conclude that the soil is not necessarily deficient in potash, but the potassium sulphate by reacting with the insoluble phosphate in the soil makes it more available to azotobacter.

Ziemiecka [1932] in her experiments with Rothamsted soils comes to the conclusion that "the azotobacter was found to develop if the soil contained at least 10 mgrm. of water-soluble phosphoric acid per kgm. of soil, but below this limit little growth occurred".

To find out how far this holds good for Pusa soils, six samples of soil from the Pot Culture House of the Chemical Section of this Institute which had received different manurial treatments and the available phosphates of which were already determined, were next subjected to the plaque test with and without K_2HPO_4 (Plate XIII, fig. 2). The following table gives the results :—

	Plot 3 N+P+K	Plot 4 N+P	Plot 6 P+K	Plot 7 N	Plot 8 P	Plot 9 K
* Mgrms. of available P_2O_5 per kilo. soil.	27	29	38	21	46	23
Control . . .	0	0	+	0	+	0
with 2·5 c.c. K_2HPO_4 .	+++ P	+++	++++ P	+++ P	++++ P	+++ P

* Figures (supplied by the Chemical Section of this Institute) refer to P_2O_5 as determined by the K_2CO_3 extraction method [Das, 1930] and not water soluble.

There was comparatively little or no growth of azotobacter in control cakes of soil from plots which had already received phosphate manuring and which on analysis showed 27 to 46 mgrm. of available phosphoric acid per kilo. of soil, whereas the addition of K_2HPO_4 gave rise to luxurious pigmented growth in all the samples.

It would appear, therefore, that the minimum phosphate requirement of azotobacter varies with the species of azotobacter that are active in different soils and the method is not sufficiently sensitive to detect the phosphate deficiencies in all soils.

Minimum dose of K_2HPO_4 necessary for appearance of azotobacter in plaques.

Since the addition of 2·5 c.c. of a 3 per cent. solution of K_2HPO_4 to 25 grms. of soil as in our tests, is equivalent to 15,280 lbs. of superphosphate per acre or nearly 40 times the amount usually recommended for a crop, an attempt was made to find out the minimum dose necessary for the appearance of azotobacter colonies. Gujranwala soil, which showed no growth in the control plaques, was employed for this test. The phosphate solution was diluted 15 times, and 0·6 c.c., 1·25 c.c. and 2·5 c.c. of this dilute solution, and also 0·5 c.c. and 2·5 c.c. of

the original solution were added in making up the cakes. The results are seen below:—

Gujranwala soil 25 grms. + 1·25 grms. of starch.

Control	With K_2HPO_4				
	1·2 mgrm.	2·5 mgrm.	5·0 mgrm.	15 mgrm.	75 mgrm. (normal dose)
0	0	2 colonies	++ P	++++ P	++++ P

Here we find that when 5·0 mgrms. of K_2HPO_4 per 25 grms. of soil or 1/15 of the normal dose used is added, colonies appear on the cakes sufficiently numerous for diagnosis and no difference in growth is observed between the normal dose of 75 mgrm. and 1/5 of the same.

Herein our results corroborate those of Jones [1932] who found that the minimum phospate requirements of azotobacter are higher than those for growing plants.

Discussion.

The azotobacter test or the soil plaque method is easy of manipulation and requires only a short time for getting results, but in the interpretation of the results, great care is necessary, as the method has many limitations in determining and distinguishing between the comparative fertilizer requirements of different soils. As has been pointed out by Ziemiecka [1932], the method cannot give indication when the soils have received nitrogenous manuring, because soluble nitrogenous compounds are detrimental to the growth of azotobacter.

Again, the method, while it depends upon the correlation of requirements of azotobacter and plant growth, does not take into account the possibility of the adaptation of strains of azotobacter to mineral deficiencies in soils. On account of this adaptation of the bacteria to their environments, the minimum requirements of potassium and phosphoric acid of the azotobacter may be different in different soils and introduce a factor of uncertainty in the interpretation.

Thus the results obtained with potassium sulphate with eight Indian soils in the experiments reported above are liable to lead to two alternative inferences depending upon the potassium requirements of the azotobacter present in the Indian soils. If the minimum requirements of azotobacter are nearly the same

as those of the crops raised in these soils, then (1) all the Indian soils examined can be said to be sufficiently rich in potash and will not respond to potassic fertilizers. On the other hand if the minimum potassium requirements of azotobacter present in Indian soils have no relation with those of the crops and are so small that they can be met even by very poor soils then the proper interpretation of the results would be (2) that the method cannot serve as a good guide for distinguishing between different soils with regard to their potassium requirements.

The exceptional instance where the addition of potassium sulphate has encouraged the growth of azotobacter in the soil from the plot manured with superphosphate can also be interpreted in two ways. (1) Either that the crops manured with superphosphate in successive years, have removed large amounts of potash owing to increased plant growth with phosphate and thus impoverished the soil of its available potash, or (2) the availability of phosphate to azotobacter depends upon associated ions like potassium, which are supplied with the addition of potassium sulphate.

It is interesting to recall that a similar exceptional instance of response to potassium sulphate was observed by Jones [1932] in a soil which had been manured with phosphate.

Although the method is of limited value for comparison of mineral deficiency of soils from different tracts because the results obtained are not easy to interpret, it may prove of great use for rapid comparative examination of a large number of fields located in the same area where the strains of azotobacter will show little variation.

The test can also be used to find out whether a soil is acid by mixing 5 per cent. starch to it and making plaques with the addition of K_2HPO_4 and noting whether any azotobacter growth occurs after incubation for 3 days at 30°C. Absence of growth indicates that the soil is acid. Inoculation of the soil with a culture of azotobacter will have to be resorted to in rare cases.

Other applications of the method.

Since it was observed in our experiments that the addition of K_2HPO_4 invariably produced a more vigorous growth of azotobacter in soils with a pH index of 6·8 and over, as compared with that of sodium phosphate, it was thought that the method would perhaps be valuable in distinguishing the availability of phosphate in different fertilizers. Four soils from Pusa, Kalyanpur, Chinsurah and Sialkot were selected to test the availability of the following substances :—

(1) Superphosphate.

(2) Solubilized bonemeal [Vyas, 1930] (Bonemeal-sulphur-charcoal-sand compost.)

(3) Apatite or Kudada phosphate.

(4) Trichy nodule.

(5) Tricalcic phosphate (Merck).

(6) Disintegrated bone-dust (prepared by treating bones with caustic soda and sodium chloride).

0·3 gm. of each fertilizer was added to 25·0 grms. of soil, which received in addition 1·25 grms. of starch. The results of observation in cakes incubated for 3 days at 30°C. are as follows :—

Substance	Pusa	Kalyanpur	Chinsurah	Sialkot
(1) Superphosphate	0F	0F	++F	++F
(2) Solubilized bonemeal	+++P	+++F	0FP	+++P
(3) Apatite	0	0	0F	0F
(4) Trichy nodule	+++	+	0F	+++P
(5) Tricalcic phosphate	+++P	++++P	+++P	++++P
(6) Bone-dust	++	+	++F	+++

Tricalcic phosphate (Merck) though insoluble, induced vigorous growth of azotobacter in all the soils tested. This is probably due to its finely pulverised nature and also to its slight solubility in water.

Kudada phosphate or apatite did not induce growth of azotobacter in any of the soils; this was expected since all its phosphate is insoluble and non-available.

Superphosphate encouraged fungus growth in all the four soils. There was azotobacter growth also in two out of the four soils used.

In another experiment, carried out with 5 different soils, using a lesser amount of superphosphate than that in the preceding one, it was found that all the five soils gave growth of azotobacter in addition to fungus. The acidity of the fertilizer used raises the hydrogen-ion concentration of the soil and this is perhaps responsible for the growth of the fungus obtained with superphosphate.

Further trials to determine the optimum quantity to be used to get growth of azotobacter without interference of fungi and also to see the effect of neutralizing the acidity of the superphosphate are in progress.

Incidentally, the fungus growth observed on the addition of superphosphate may explain the encouragement of fungal attacks of crops in some soils manured

with superphosphate. Moreover, the fungi would utilize the soluble phosphate for their growth and convert it into some organic form which may not be immediately available to plants.

Solubilized bonemeal seems to serve as a good nutrient for azotobacter in 3 soils ; in Chinsurah soil, which is clayey, it encouraged fungus growth only. Chinsurah soil appears to have a predominating fungus flora as will be seen from the table and the rapidity of the growth of the natural fungi might have suppressed the appearance of azotobacter colonies in this soil.

Trichy nodule gave good growth of azotobacter in Pusa and Sialkot soils, scanty growth in Kalyanpur soil and only fungus growth in Chinsurah soil, showing that when finely ground it is available for azotobacter, a conclusion which is in agreement with its availability for crops as found by Ramaswami Sivan [1922].

Addition of bone-dust prepared by treating bones with caustic soda and common salt, produced growth of azotobacter in all the soils, but the colonies appeared later in the plaques than in other cases, showing that probably it is available more slowly than the other fertilizers.

The above facts suggest that the plaque test is likely to prove useful in quickly determining the extent to which different phosphatic fertilizers would be available in any particular soil.

Summary and conclusions.

(1) The azotobacter plaque method according to Sackett's technique has been applied to eight Indian soils.

(2) Until the minimum quantities of potassium and phosphoric acid necessary for the growth of azotobacter are correlated with the needs of these minerals to plants in the soils to be tested, great care will have to be exercised in interpreting the results.

(3) It is indicated that the plaque method may prove useful in distinguishing between the availability of different phosphatic fertilizers.

(4) There is also a possibility of the method being useful in testing the acidity of a soil and finding out its lime requirements.

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SOME EXPERIMENTS ON THE CONTROL OF LOOSE SMUT, *USTILAGO TRITICI* (PERS.) JENS., OF WHEAT.

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(With two text-figures)

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I. Introduction.

Loose smut caused by the fungus *Ustilago tritici* (Pers.) Jens. is one of the most well known and destructive diseases of wheat. It occurs in almost every part of the punjab to a more or less extent. Generally, the damage caused by the disease is slight and therefore no notice is taken of it. But in 1923, a serious attack of the disease amounting to 30 per cent. in some cases was observed in the Upper and Lower Jhelum canal colonies. Enquiries made in other parts of the Punjab also showed that the attack of the disease in several districts was serious enough to

require control. For example, wheat fields in Kangra, Hoshiarpur, Gurdaspur, Amritsar, Sialkot, Gujrat, Rawalpindi, Karnal and Ambala were affected up to 10 per cent. or more with this disease. In the Punjab, wheat occupies an area of about 10 million acres every year, and if on an average of the whole Province the damage caused by the disease is taken as low as two per cent., the total economic loss sustained through this disease works out at nearly fifty lakhs of rupees (about £400,000) annually. But what matters most are the heavy losses of those individual zamindars in whose fields the disease occurs to the extent of 20 per cent. or even more. Such cases were very alarming and made it imperative for the Department of Agriculture to undertake experiments for the control of the disease. As the life-history and the mode of infection of the loose smut fungus have been fully worked out and considerable research on the methods of control also has already been carried out, the work of the authors was much facilitated in planning out such simple methods of treatment as would be practicable for adoption by farmers. Before describing details of the experiments carried out for this purpose, a brief reference is made below to the work done on the control of this disease in other countries :—

Jensen [1888] was the first to demonstrate that loose smut of wheat could be prevented by subjecting the seed wheat, before sowing, to hot water treatment. According to his method, wheat is soaked in water at ordinary temperature for four hours; then it is dipped in water at 132° F. for 10 minutes; and finally it is dried before sowing. Jensen's method has undergone several modifications and the procedure of the hot water method now applied is as follows :—

(1) Wheat is soaked in water at 68° to 86°F. for 4 to 6 hours, and (2) dipped in water heated to 120°F. for two minutes; then (3) it is immersed in water at 129°F. for 10 minutes; finally (4) it is dried before sowing.

Although this method is quite effective for controlling the disease, as reported by Freeman and Johnson [1909] and many others, yet it was not practised commonly in U. S. A. until the 'community system' described by Pipal [1921] was devised. Tapke [1924] reports that though in certain community centres in U. S. A., the method is very much in use, yet in several places it has not been adopted, because of certain defects, for example, the germination capacity of the seed is injured by heating, especially when there are many broken grains. It is also tedious and requires much care to maintain the temperature constant at 129°F. More recently Tapke [1926] brought out single bath hot water and steam treatments. By these methods, although germination power of the seed is not affected, yet as elaborate machinery is required, they have not become popular.

To meet the situation in the Punjab, demonstrations were given to explain the hot water treatment and it was ultimately tried on various Agricultural Farms. Seed wheat was sown after being treated and many places were rendered free of the disease by the supply of smut-free seed. Although the method was successful in eliminating the desease in badly affected localities, it could not be taken up as a general measure of control, as only small quantities of seed could be dealt with at a time. Moreover, the method could be applied only under expert guidance. A large number of trained and literate hands were needed to treat wheat for zamindars who, being illiterate, can not read a thermometer and maintain the required temperature. In view of these serious drawbacks, simplification of the method was felt absolutely necessary, especially with regard to the mode of maintaining the lethal temperature constant for the required time. With these objects in view, experiments were conducted with regard to (1) the simplification of the recognised hot water treatment method, (2) single-bath treatments, (3) the application of solar energy for heating water and soaking of the wheat in it, and (4) the application of solar energy by exposure of wheat already soaked in water.

II. Experimental.

The wheat used in all the experiments was "Punjab 8-A" — a selection of the Botanical Section, Lyallpur, introduced in the Punjab as improved wheat, unless otherwise mentioned. Wheat required for the experiments was obtained from fields around Sargodha, where, on the average, the smut disease in the crop was about 10 per cent. For heating water, small heaters called '*hamams*' were used, and for treating the seed, galvanised iron tubs of 38 inches bottom measurement were employed. Seed wheat after treatment was always dried in the sun for 5-6 hours.

The germination tests of treated as well as untreated samples were carried out in the usual manner.

In all the field experiments every sample was sown in 1/200th of an acre plot. In all cases alternate control plots were arranged, and the experiments were run in triplicate.

The percentage of smut wherever given in the following experiments is based on a count of 500-1,000 plants.

A. Simplification of the hot water treatment method.

(*1*) *Final hot water immersion.*

Experiment (*i*).—It was thought first of all to determine whether it was necessary to maintain a constant temperature of 129°F. for the third stage of the treat-

ment. All the seed used was presoaked in water at room temperature (65°-70°F.) for 4 hours, and dipped in hot water at 120°F. for 2 minutes. The seed was then divided into 8 lots each of which was immersed in water at different temperatures for 2-10 minutes (Table I). The seed grains after treatment were dried and the germination capacity of a sample determined. The remainder was sown in the field and observations on the occurrence of smut were recorded.

Table I shows the effect of different treatments on the germination capacity of the seed and on the control of smut.

It will be seen that steeping for 10 minutes in water at temperatures ranging from 127°-132°F. does not materially affect the viability of the seed. Soaking of wheat in water at a temperature above 135° F. for more than 2 minutes injures the seed. All the treatments, however, effectively controlled smut. The results clearly show that it is not necessary to have a constant temperature at 129° F. for 10 minutes. Consequently in the case of the Punjab wheat Type 8-A the temperature of water at the third stage of the treatment may be allowed to vary between 127° and 132° F., without losing efficacy to kill the fungus in the grain.

TABLE I.

Serial No.	Treatment	1927-28			1928-29		
		Date of treatment	Average percentage of germination	Average percentage of smut	Date of treatment	Average percentage of germination	Average percentage of smut
1	140° F. for 5 minutes	20th November 1927.	...	...	13th November 1928.	18	0
2	135° F. „ 10 „	20th November 1927.	...	...	13th November 1928.	49	0
3	135° F. „ 5 „	20th November 1927.	90	0	13th November 1928.	69	0
4	135° F. „ 2 „	20th November 1927.	96	0	13th November 1928.	99	0
5	132° F. „ 10 „	20th November 1927.	94	0	13th November 1928.	90	0
6	130° F. „ 10 „	20th November 1927.	98	0	13th November 1928.	91	0
7	129° F. „ 10 „	20th November 1927.	95	0	13th November 1928.	91	0
8	127° F. „ 10 „	20th November 1927.	97	0·06	13th November 1928.	96	0
9	Untreated . .	20th November 1927.	99	7·8	13th November 1928.	98	4·5

Experiment (*ii*).—The period of two minutes at the second step is too short to keep up and inconvenient for treating large quantities of wheat. In the following experiments wheat soaked for 4 hours was dipped in water at 120° F. and kept there for 5 minutes. It was then divided into 6 lots and each of them was immersed in hot water at a temperature ranging between 127° and 132° F. for 2, 4, 6, 7, 8 and 10 minutes respectively. For the final immersion, 132° F. was chosen as the next safe temperature in view of the results of the experiment described at (*I*) (*i*) above. Moreover, the temperature was not allowed to fall below 127° F. by letting in warmer water from the heater if necessary. Results of the experiments are given in Table II.

TABLE II.

Serial No.	Time of hot water immersion (127°-132° F.)	1928-29			1929-30		
		Date of treatment	Average percentage of germination	Average percentage of smut	Date of treatment	Average percentage of germination	Average percentage of smut
1	2 minutes . .	11th November 1928.	98	4·7	14th November 1929.	99	7·6
2	4 „ . .	11th November 1928.	98	2·9	14th November 1929.	98	3·1
3	6 „ . .	11th November 1928.	97	0·1	14th November 1929.	98	0·2
4	7 „ . .	11th November 1928.	96	0	14th November 1929.	96	0
5	8 „ . .	11th November 1928.	96	0	14th November 1929.	95	0
6	10 „ . .	11th November 1928.	94	0	14th November 1929.	93	0
7	Untreated . .	11th November 1928.	98	6·4	14th November 1929.	99	11·4

The table shows that germination capacity is not injured to any appreciable extent except in the case of treatment No. 6 (ten minutes immersion), where it is reduced by about 5 per cent. The result of the experiments further show that for a complete control of the disease, presoaked wheat dipped in water at 120° F. for 5 minutes instead of 2 minutes as usual should be immersed in water at a temperature that may vary between 127° and 132° F. for a minimum time of 7 minutes. The margin of 5° F. makes the process more handy.

(2) Presoaking.

An experiment was devised to find out the maximum and minimum time for which the seed wheat should be presoaked in water at the ordinary room temperature of 65°-70° F. before it is subjected to the next stage of the treatment. Eight lots of seed wheat were presoaked in water at 65°-70° F. for periods varying from 1-8 hours. The subsequent treatment given was the same as in the concluding portion of paragraph (*1, ii*) above. The results of germination tests and the effect of different treatments on the control of smut are shown in Table III.

TABLE III.

1928-29.

Serial No.	Date of treatment	Time of soaking	Average percentage of germination	Average percentage of smut
1	12th November 1928.	1 hour	98	3·3
2		2 hours	95	1·7
3		3 ,,	98	6·6
4		4 ,,	97	0
5		5 ,,	95	0
6		6 ,,	94	0
7		7 ,,	93	0
8		8 ,,	90	0
9		Untreated	98	6·0

From the results contained in the above table it is clear that presoaking of seed wheat in water at 65°-70°F. for 4-7 hours prior to immersion in hot water in steps Nos. (2) and (3) gives a complete control of smut without materially affecting the germination of the seed.

(3) *Storage of treated wheat.*

Wheat treated with the simplified method described under (4) was dried thoroughly in the sun for two days and stored for various periods. Germinating capacity of seed grains was determined. The results are given below in Table IV.

TABLE IV.

1929-30.

Serial No.	Date of treatment 1929	Date of sowing for germination test 1929	Period of storage	Average percentage of germination
1	10th September	14th November	9 weeks	95
2	2nd October	14th November	6 „	94·5
3	22nd October	14th November	3 „	95
4	14th November	14th November	*Nil.*	95
5	Untreated	14th November	....	99

It would appear from Tables II, III and IV that the hot water treatment reduces the germination capacity of treated wheat by about 5 per cent. Table IV shows that the germination of treated seed is not further reduced as a result of storage up to 9 weeks.

(4) *Description of the 'simplified method of hot water treatment'.*

On the basis of the results of the foregoing experiments (1), (2) and (3) a very convenient and simple method of hot water treatment has been evolved. It is described below along with the equipment required for it.

Equipment.—(1) Two heaters locally called '*hamams*' (Fig. 1) each with a capacity of about 35 gallons of water.

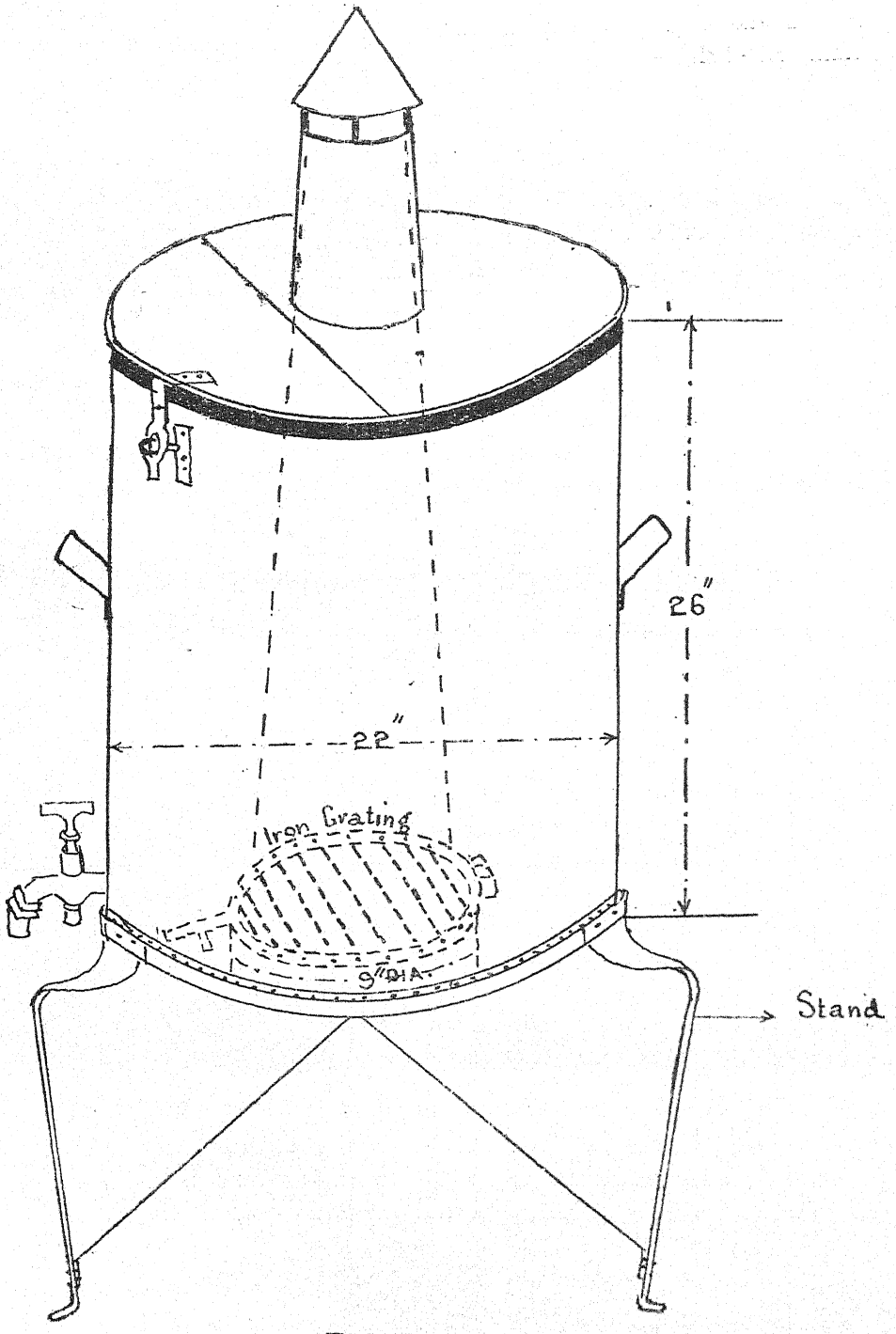


Fig. 1.—*Hamam* or heater.

(2) Two galvanized iron tubs (Fig. 2) each with a bottom measurement of 38 inches, and having level marks on the sides at 25 gallons of water.

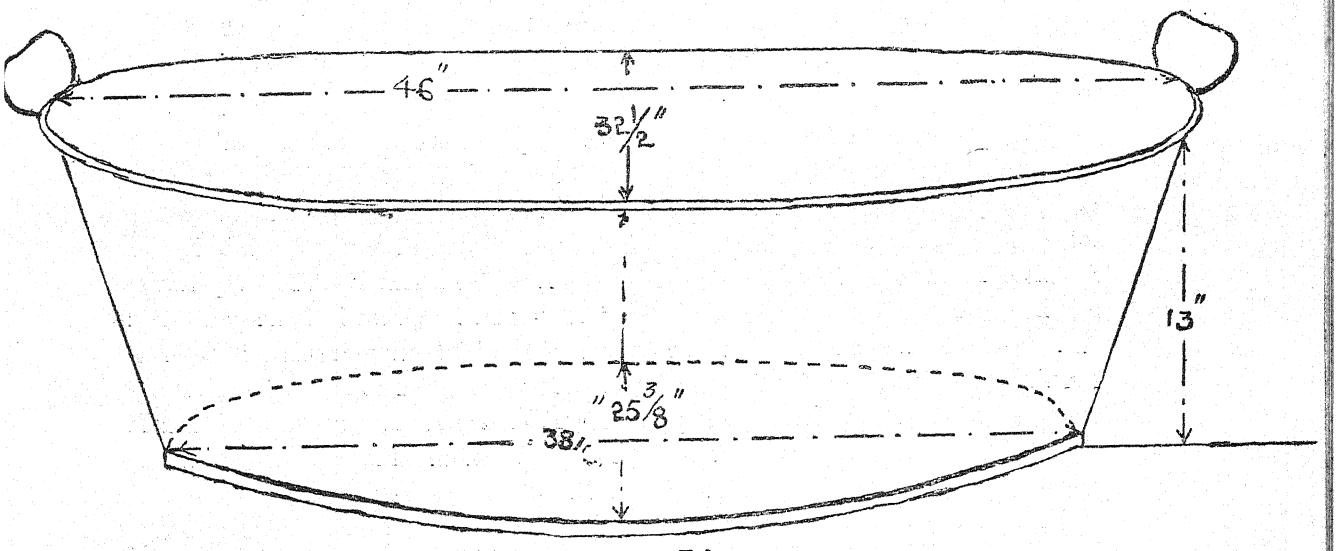


Fig. 2.—Tub.

(3) A big tub or a vessel of any other kind to be used for presoaking of wheat.

(4) Two sheets of coarse cotton cloth measuring about 7×5 feet.

(5) One stirrer preferably a small vessel.

(6) One Fahrenheit thermometer.

(7) One or more sheets of cloth of suitable size on which wheat is to be spread for drying after treatment.

Method.—The two heaters (*hamams*) with the two standardized tubs, which may be designated as No. 1 and No. 2 are placed (opposite to one another) in a well ventilated room. Charcoal is used to heat water in the heaters (*hamams*), and the temperature of water is brought near to the boiling point before the treatment is commenced. During the course of the treatment draught should be avoided to prevent rapid fall of temperature of water in the tubs.

Two and a half maunds of wheat are soaked in water in the morning at about 7 o'colck for four hours, if the treatment is carried out in September, when the temperature in an ordinary room will be nearly 85°F. But if wheat is to be

treated in October or November, it should be soaked for 5 and 7 hours respectively, as the temperature falls to 70° and 60°F. In warm weather water soaks into the grain more quickly than under colder conditions. As the temperature in October and November is lower, more time has to be given to let water penetrate into the grain properly. The soaked wheat is taken out and divided into nearly five equal lots.

Then about 10 gallons of ordinary water are put in each of the two tubs, and hot water from the heater (*hamam*) is run into them until the level of water is brought upto the 25 gallon mark and the thermometer shows 132°F. Then one lot of the soaked wheat carried in a cloth sheet, is dipped in water in tub No. 1 for five minutes. The grains are shaken by means of a stirrer. The temperature of the water after stirring will fall to about 118°-122°F. Wheat is taken out, and immersed for 7 minutes in tub No. 2, where the initial temperature of water is 132°F. The grains are stirred and the temperature will fall to about 130°F. and if it goes down further it should be kept at 127°F. or above but not beyond 132°F. during the required immersion time of 7 minutes by adding hot water, but if the temperature does not fall below 127°F., no hot water need be added.

Wheat is taken out, and spread out in the sun on a cloth sheet to dry. The other 4 lots of wheat are treated in the same way successively. By this method 10 maunds of seed wheat can be conveniently treated in a day of 8 hours with one set of equipment.

The treated seed wheat after drying can be sown immediately, or if desired, can be stored for 2 months at least, but thorough drying is essential previous to storing to protect the seed from mould fungi.

As the germination of seed grains is reduced by about 5 per cent. on account of heat, it is recommended that the seed rate of treated wheat should be increased by 5 per cent. in order to get a normal stand of the crop.

This method of treatment has been tried for five years and is now practised with complete success on all Agricultural Farms, wherever loose smut of wheat is appreciable.

B. Single-bath treatments at different temperatures.

Although the usual hot water treatment method as described in the previous pages has been somewhat simplified, yet if it could be made still easier, particularly with regard to the elimination of some stages of the process, its utility as a practi-

cal measure of control will greatly increase. With this object in view, the following further experiment was conducted :—

Seed wheat was soaked in water at (*i*) 100°F., (*ii*) 105°F., (*iii*) 110°F., (*iv*) 115°F., for 2, 4, 6 and 8 hours. These temperatures were kept constant for the required period by adding hot water from a heater from time to time. The seed grains thus treated were dried and the germinating capacity of the samples was tested. Samples were also sown in the field in order to find out the effect of these treatments on the control of smut. The results given in Table V show that the germination of seed grain is not impaired by any of the treatments, and that the soaking of seed wheat in water at 105°F., 110°F. and 115°F. for 8, 6 and 4 hours respectively, has been effective in the control of the smut.

TABLE V.

1929-30.

1	2	3	4		5		6		7	
Serial No.	Date of treatment	Temperature of water at which soaked	Soaked for 2 hours		Soaked for 4 hours		Soaked for 6 hours		Soaked for 8 hours	
			Percentage of germination	Percentage of smut	Percentage of germination	Percentage of smut	Percentage of germination	Percentage of smut	Percentage of germination	Percentage of smut
1	18th November 1929	100°F. .	98	6·2	98	6·2	96	1·6	99	3·4
2		Untreated .	98	6·4	97	8·0	97	7·0	96	8·8
3	18th November 1929	105°F. .	98	4·9	98	3·2	99	1·9	99	0
4		Untreated .	98	7·3	97	6·1	97	5·2	96	7·2
5	18th November 1929	110°F. .	99	4·8	98	0·4	97	0	96	..
6		Untreated .	98	6·6	97	7·6	97	5·8	96	6·9
7	18th November 1929	115°F. .	99	8·9	96	0	96	0	96	0·03*
8		Untreated .	98	10·4	97	10·0	97	10·2	96	10·9

* This is negligible.

A careful examination of the table will show that if water at 115°F. is let into the tub and even if its temperature falls to 105°F., it can be used effectively against

smut provided wheat is immersed into it for eight hours and water is kept at that temperature by addition of hot water from the heater. For temperatures above 105°F., the period of immersion should vary as given in the table. This method although effective appears to be somewhat difficult to carry out, as the maintenance of constant temperature for several hours requires much attention and patience.

C. Single-bath sun-heated water method.

Having established in the experiment described above that a single immersion in water at temperatures 105°, 110° and 115°F. for 8, 6 and 4 hours respectively, is effective to control smut, an idea arose that heating of water might be done by the application of solar energy. Sun in the Punjab plains is very strong in summer and the intensity of heat which prevails daily from 10 a. m. to 5 p. m. is a source of great potential energy. Temperature in the shade rises to 120°F. and directly in the sun it is as high as 131°F. It was thought that this natural heating power might be availed of for the purpose of smut control. The experiments conducted in this connection are detailed below :—

A galvanized iron vessel 30 inches high and 18 inches in diameter, which was found suitable for the experiments after trials was half filled with water and placed in the sun in the morning at 8 o'clock on bright sunny days in summer from June to August at Lyallpur. The seed wheat was added at 12 noon. Samples were taken out after an interval of 4 and 5 hours and dried and then stored till these were sown in the field in November. The stand of the crop raised from treated and untreated seed was equally good. The crop was examined for the determination of percentage of smut when it reached maturity. The results are given in Table VI.

TABLE VI.

Serial No.	Type of wheat	Date of treatment	Duration of treatment	Temperature of sun-heated water in Fahrenheit at				Maximum temperature in Fahrenheit under shade	Percentage of germination	Percentage of smut
				12 noon	2 p.m.	4 p.m.	5 p.m.			
1	2	3	4	5	6	7	8	9	10	11
1	Pb. 8A	11th June 1931 .	4 hrs. (12—4) . .	100	112	117	..	114·2	97	0
	,,	Untreated . .	....	..	..	..	..	..	100	3·05
2	Pb. 8A	11th June 1931 .	5 hrs. (12—5) . .	100	112	117	119	114·2	97	0
	,,	Untreated . .	....	..	..	..	..	..	100	4·16
3	Pb. 8A	12th June 1931 .	4 hrs. (12—4) . .	102	113	115	..	112·5	98	0
	,,	Untreated . .	....	..	..	..	..	..	100	3·52
4	Pb. 8A	12th June 1931 .	5 hrs. (12—5) . .	102	113	115	112	112·5	100	0
	,,	Untreated . .	....	..	..	..	..	..	100	4·08
5	Pb. 8A	17th June 1931 .	4 hrs. (12—4) . .	95	107·5	110	..	96·0	99	·30
	,,	Untreated . .	....	..	..	..	..	..	100	5·14
6	Pb. 8A	17th June 1931 .	5 hrs. (12—5) . .	95	107·5	110	111	96·0	100	·69
	,,	Untreated . .	....	..	..	..	..	..	100	6·29
7	Pb. 8A	18th June 1931 .	4 hrs. (12—4) . .	100·2	112	118	..	102·7	99	0
	,,	Untreated . .	....	..	..	..	..	..	100	5·66

TABLE VI—*contd.*

Serial No.	Type of wheat	Date of treatment	Duration of treatment	Temperature of sun-heated water in Fahrenheit at				Maximum temperature in Fahrenheit under shade	Percentage of germination	Percentage of smut
				12 noon	2 p.m.	4 p.m.	5 p.m.			
1	2	3	4	5	6	7	8	9	10	11
8	Pb. 8A	18th June 1931 .	5 hrs. (12—5) . .	100·2	112	118	119	102·7	98	0
	„	Untreated . .	....	..	..	..	..	..	100	4·24
9	Pb. 8A	20th June 1931 .	4 hrs. (12—4) . .	99	108	111·5	..	101·6	96	0
	„	Untreated . .	....	..	..	..	..	..	100	5·55
10	Pb. 8A	20th June 1931 .	5 hrs. (12—5) . .	99	108	111·5	114	101·6	95	0
	„	Untreated . .	....	..	..	..	..	..	100	4·40
11	Pb. 8A	21st June 1931 .	4 hrs. (12—4) . .	97·5	109	112	..	104·8	100	0
	„	Untreated . .	....	..	..	..	..	..	100	8·11
12	Pb. 8A	21st June 1931 .	5 hrs. (12—5) . .	97·5	109	112	114	104·8	99	0
	„	Untreated . .	....	..	..	..	..	..	100	6·66
13	Pb. 8A	23rd June 1931 .	4 hrs. (12—4) . .	103	111	116	..	110·0	100	0
	„	Untreated . .	....	..	..	..	..	..	100	4·42
14	Pb. 8A	23rd June 1931 .	5 hrs. (12—5) . .	103	111	116	114	110·0	99	0
	„	Untreated . .	....	..	..	..	..	..	100	7·50
15	Pb. 8A	24th June 1931 .	5 hrs. (12—5) . .	100	107	111	114	110·3	99	0
	„	Untreated . .	....	..	..	..	..	..	100	6·19

16	Pb. 8A	29th June 1931 .	4 hrs. (12—4) . .	104	Not recorded.	118	..	114·2	99	0
	,,	Untreated . .	....	..	..	..	..	..	100	8·20
17	Pb. 8A	29th June 1931 .	5 hrs. (12—5) . .	104	Not recorded.	118	119·5	114·2	100	0
	,,	Untreated . .	....	..	..	..	..	..	100	7·39
18	Pb. 4	30th June 1931 .	5 hrs. (12—5) . .	103	Not recorded.	115	116·5	114·2	98	0
	,,	Untreated . .	....	..	..	..	..	..	100	9·64
19	Pb. 4	13th July 1931 .	4 hrs. (12—4) . .	101	108	113	..	102·5	100	0
	,,	Untreated . .	....	..	..	..	..	..	100	5·64
20	Pb. 4	13th July 1931 .	5 hrs. (12—5) . .	101	108	113	114	102·5	98	0
	,,	Untreated . .	....	..	..	..	..	..	100	10·0
21	Pb. 4	14th July 1931 .	4 hrs. (12—4) . .	99	108	112	..	103·9	97	0
	,,	Untreated . .	....	..	..	..	..	..	100	6·54
22	Pb. 4	14th July 1931 .	5 hrs. (12—5) . .	99	108	112	113	103·9	96	0
	,,	Untreated . .	....	..	..	..	..	..	100	6·09
23	Pb. 4	15th July 1931 .	4 hrs. (12—4) . .	101	Not recorded.	112	..	100·0	99	0
	,,	Untreated . .	....	..	..	..	..	..	100	9·12
24	Pb. 4	15th July 1931 .	5 hrs. (12—5) . .	101	Not recorded.	112	113	100·0	99	0
	,,	Untreated . .	....	..	..	..	..	..	100	6·09
25	Pb. 4	16th July 1931 .	4 hrs. (12—4) . .	101·5	109	116	..	102·5	88	0
	,,	Untreated . .	....	..	..	..	..	..	100	7·75

TABLE VI—*contd.*

Serial No.	Type of wheat	Date of treatment	Duration of treatment	Temperature of sun-heated water in Fahrenheit at				Maximum temperature in Fahrenheit under shade	Percentage of germination	Percentage of smut
				12 noon	2 p.m.	4 p.m.	5 p.m.			
1	2	3	4	5	6	7	8	9	10	11
26	Pb. 4	16th July 1931 .	5 hrs. (12—5) . .	101·5	109	116	116	102·5	92	0
	,,	Untreated . .	....	..	..	..	..	..	100	10·52
27	Pb. 4	17th July 1931 .	4 hrs. (12—4) . .	103·5	Not recorded.	112	..	103·9	98	0
	,,	Untreated . .	....	..	..	..	..	..	100	5·00
28	Pb. 4	17th July 1931 .	5 hrs. (12—5) . .	103·5	Not recorded.	112	112·5	103·9	93	0
	,,	Untreated . .	....	..	..	..	..	..	100	7·77
29	Pb. 4	1st August 1931 .	4 hrs. (12—4) . .	102	Not recorded.	112	..	95·0	98	·39
	,,	Untreated . .	....	..	..	..	..	..	100	7·46
30	Pb. 4	1st August 1931 .	5 hrs. (12—5) . .	102	Not recorded.	112	113	95·0	99	·49
	,,	Untreated . .	....	..	..	..	..	..	100	7·51
31	Pb. 4	6th August 1931 .	4 hrs. (12—4) . .	104·5	Not recorded.	113	..	103·0	98	0
	,,	Untreated . .	....	..	..	..	..	..	100	8·92
32	Pb. 4	6th August 1931 .	5 hrs. (12—5) . .	104·5	Not recorded.	113	114·5	103·0	100	0

	„	Untreated . .	....	..	..	..	..	..	100	7·62
33	Pb. 4	10th August 1931	4 hrs. (12—4) . .	105	Not recorded.	113·5	..	101·0	96	0
	„	Untreated . .	....	..	..	..	..	..	100	8·05
34	Pb. 4	10th August 1931	5 hrs. (12—5) . .	105	Not recorded.	113·5	114·5	101·0	97	0
	„	Untreated . .	....	..	..	..	..	..	100	7·69
35	Pb. 4	11th August 1931	4 hrs. (12—4) . .	102	Not recorded.	113	..	102·0	96	0
	„	Untreated . .	....	..	..	..	..	..	100	10·76
36	Pb. 4	11th August 1931	5 hrs. (12—5) . .	102	Not recorded.	113	114	102·0	100	0
	„	Untreated . .	....	..	..	..	..	..	100	8·78
37	Pb. 4	9th June 1932 .	4 hrs. (12—4) . .	104	112·5	120	..	112	100	0
38	Pb. 4	11th June 1932 .	4 „ (12—4) . .	106	115	120	..	113	100	0
39	Pb. 4	12th June 1932 .	4 „ (12—4) . .	110	117	123	..	116	99	0
	„	Untreated . .	....	..	..	..	..	..	100	15·39
40	Pb. 4	13th June 1932 .	4 hrs. (12—4) . .	107	115	121	..	116	99	0
41	Pb. 4	16th June 1932 .	4 „ (12—4) . .	105	114	118	..	109	100	0
42	Pb. 4	17th June 1932 .	4 „ (12—4) . .	106	114·5	119	..	108	100	0
43	Pb. 4	18th June 1932 .	4 „ (12—4) . .	106	114	117	..	109	100	0
44	Pb. 4	19th June 1932 .	4 „ (12—4) . .	108·5	116	119	..	113	99	0
45	Pb. 4	23rd June 1932 .	4 „ (12—4) . .	105	110·5	115·5	..	97	100	·86
46	Pb. 4	24th June 1932 .	4 „ (12—4) . .	104	113	118	..	104	100	0
	„	Untreated . .	....	..	..	..	..	..	100	16·26

TABLE VI—*contd.*

Serial No.	Type of wheat	Date of treatment	Duration of treatment	Temperature of sun-heated water in Fahrenheit at				Maximum temperature in Fahrenheit under shade	Percentage of germination	Percentage of smut
				12 noon	2 p.m.	4 p.m.	5 p.m.			
1	2	3	4	5	6	7	8	9	10	11
47	Pb. 4	27th June 1932	4 hrs. (12—4) . .	107	117	121	..	112	100	0
48	Pb. 4	28th June 1932 .	4 „ (12—4) . .	107·7	116·5	122	..	113	100	0
49	Pb. 4	5th July 1932 .	4 „ (12—4) . .	107·5	116	122	..	111	100	0
50	Pb. 4	6th July 1932 .	4 „ (12—4) . .	105·7	113	118	..	113	99	0
	„	Untreated . .	....	..	..	..	..	..	100	14·88
51	Pb. 8A	8th July 1932 .	4 hrs. (12—4) . .	106	114·5	118·5	..	114	99	0
	„	Untreated . .	....	..	..	..	..	..	100	10·77
52	Pb. 4	29th August 1932	4 hrs. (12—4) . .	99	107	114	..	96	100	·51
53	Pb. 4	30th August 1932	4 „ (12—4) . .	102	108	115	..	100	100	·50
54	Pb. 4	31st August 1932	4 „ (12—4) . .	101	109	115·5	..	103	100	0
55	Pb. 4	1st September 1932	4 „ (12—4) . .	102	109·5	116	..	104	100	0
56	Pb. 4	3rd September 1932	4 „ (12—4) . .	103	110	116	..	107	100	0
	„	Untreated . .	....	..	..	..	..	..	100	14·21

The results show that the smut disease is controlled effectively by single step treatment and the germination of treated grains is not impaired by any of the treatments. It has also been found that such treated wheat does not deteriorate in its germinating power if it is stored.

D.—Exposure of pre-soaked wheat to the sun.

Various experiments were laid out in which samples of smutted seed wheat of different types were pre-soaked in water at ordinary temperature for four hours, *i.e.*, 8 a.m. to 12 noon; then taken out and exposed to the sun from 12 noon to 4 p.m. on different days in June and July. These were then sown in the field in November. The results are given in Table VII.

TABLE VII.

Serial No.	Type of wheat	Date of treatment	Duration of soaking in unheated water	Duration of exposure in the sun	Temperature in degrees Fahrenheit in the sun at			Maximum temperature in Fahrenheit under shade	Percentage of germination	Percentage of smut
					12 noon	2 p.m.	4 p.m.			
			A.M.	P.M.						
1	Pb. 8A	10th June 1931	4 hrs. (8—12) . .	4 hrs. (12—4) . .	122	126	129	114·2	100	0
	,,	Untreated .	....	....	..	..	..	..	100	4·36
2	Pb. 4	10th June 1931	4 hrs. (8—12) . .	4 hrs. (12—4) . .	122	126	129	114·2	100	0
	,,	Untreated .	....	....	..	..	..	..	100	8·02
3	Pb. 8A	11th June 1931	4 hrs. (8—12) . .	4 hrs. (12—4) . .	121	124	125	112·5	100	0
	,,	Untreated .	....	....	..	..	..	..	100	4·31
4	Pb. 4	11th June 1931	4 hrs. (8—12) . .	4 hrs. (12—4) . .	121	124	125	112·5	100	0
	,,	Untreated .	....	....	..	..	..	..	100	8·97
5	Pb. 4	10th June 1932	4 hrs. (8—12) . .	4 hrs. (12—4) . .	122	122	122·5	115	99	0
	,,	Untreated .	....	....	..	..	..	..	100	13·16
6	Pb. 4	11th June 1932	4 hrs. (8—12) . .	4 hrs. (12—4) . .	122	123	125	113	98	0
	,,	Untreated .	....	....	..	..	..	..	100	13·09

7	Pb. 4	12th June 1932	4 hrs. (8—12) . .	4 hrs. (12—4) . .	123	125·5	124	116	99	0
	,,	Untreated .	....	....	..	..	..	..	100	11·95
8	Pb. 4	18th June 1932	4 hrs. (8—12) . .	4 hrs. (12—4) . .	122	124·5	122	109	98	0
9	Pb. 4	27th June 1932	4 hrs. (8—12) . .	4 hrs. (12—4) . .	121	125·5	125·5	112	99	0
	,,	Untreated .	....	....	..	..	..	..	100	13·95
10	Pb. 4	28th June 1932	4 hrs. (8—12) . .	4 hrs. (12—4) . .	121	129	126	113	99	·26
	,,	Untreated .	....	....	..	..	..	..	100	13·04
11	Pb. 8A	28th June 1932	4 hrs. (8—12) . .	4 hrs. (12—4) . .	121	129	126	113	99	0
	,,	Untreated .	....	....	..	..	..	..	100	9·70
12	Pb. 4	5th July 1932 .	4 hrs. (8—12) . .	4 hrs. (12—4) . .	124·5	125·5	125	111	99	0
13	Pb. 4	6th July 1932 .	4 hrs. (8—12) . .	4 hrs. (12—4) . .	123	125·5	124·5	113	98	0
	,,	Untreated .	....	....	..	..	..	..	100	14·96
14	Pb. 8A	6th July 1932 .	4 hrs. (8—12) . .	4 hrs. (12—4) . .	123	125·5	124·5	113	98	0
	,,	Untreated .	....	....	..	..	..	..	100	11·25
15	Pb. 8A	27th June 1932	4 hrs. (8—12) . .	4 hrs. (12—4) . .	121	125·5	125·5	112	98	0
	,,	Untreated .	....	....	..	..	..	..	100	10·85

It is clear from the results that if the seed wheat is soaked in water for four hours (8 a.m. to 12 noon) and then exposed to the sun (from 12 noon to 4 p.m.), the smut is controlled and the germination power of the seed grains does not suffer.

III. Conclusions.

The simplified method of hot water treatment has been tried for the last five years with complete success on all the Agricultural Farms in the Punjab, where loose smut of wheat is a matter of importance. It will certainly be found applicable to other wheat growing tracts of India.

Although this method is fairly simple, yet as it requires the use of a thermometer, and demands some skill to maintain the required temperature of water, it can only be recommended to literate farmers.

The single-bath, sun-heated water and solar energy methods (exposure of pre-soaked wheat to the sun) are devised here for the first time. In these methods the cost of treatment is reduced to a negligible amount and the use of a thermometer is dispensed with. The methods are very simple and can be recommended to even illiterate farmers of the Punjab.

These solar energy methods are, at present, applicable only to hotter parts of the plains of the Punjab. For the hilly tracts, the simplified hot water method only can be recommended.

The authors have pleasure to acknowledge the help rendered by S. Kishan Singh Bedi, B. Sc. (Ag.), during the course of this investigation.

IV. Summary.

The loose smut of wheat occurs commonly in the Punjab and in some parts the damage caused by it is as high as 10-20 per cent.

Simplified hot water treatment, single-bath method and two other new methods in which solar energy is used for heating water are described in the paper. A brief outline of the methods evolved is given below:—

1. Simplified hot water treatment method.
   - (*i*) Wheat is pre-soaked in unheated water at 60°-85° F. for 4-7 hours.
   - (*ii*) It is then dipped in water at 120° F. for 5 minutes.
   - (*iii*) Finally it is immersed in water at 127°-132° F. for 7 minutes.
   - (*iv*) It is then thoroughly dried and sown immediately or can be stored till sowing time.

2. Single-bath method.

Soaking of seed wheat in water at 105° F., 110° F., and 115° F. for 8, 6 and 4 hours respectively gives a complete control of the smut.

3. Sun-heated water method.

A blackened cylindrical galvanised iron vessel, 30 inches long and 16 inches in diameter half filled with water, is placed in the sun in the morning on any bright sunny day in summer and seed wheat is soaked in it from 12 noon to 4 p.m. and then dried.

4. Solar energy method (exposure of pre-soaked wheat to the hot sun).

Seed wheat is soaked in water for four hours (8 a.m. to 12 noon) on any bright summer day; then taken out and exposed to the sun for four hours (12 noon to 4 p.m.). Maximum temperature in shade at Lyallpur goes up to 120° F. and in the sun it has been recorded up to 131° F. in June.

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THE LIFE-HISTORY AND SEASONAL ABUNDANCE OF THE VESICATING BEETLE, *PAEDERUS FUSCIPES* CURT.

BY

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(Received for publication on 5th September 1933)

(With Plate XIV)

INTRODUCTION.

Although *Paederus fuscipes* Curt. is found in many parts of the world including Europe, New Guinea, Australia and Asia and is of medical importance as being the cause of a vesicular dermatitis in man known commonly in India as "spider lick", there is no record of its breeding habits or pre-adult stages. The original description of the species by Curtis [1826] is based on a specimen collected in England from amongst lichen in the New Forest near Brockenhurst. In a previous paper, the writer [Isaac, 1933] stated that these beetles are found generally in hiding under vegetation along the banks of rivers and permanent water channels.

BREEDING GROUNDS.

This beetle breeds in damp porous soil, rich in decaying matter, along the banks of rivers, irrigation channels, drains and pools, a few inches above the edge of the water. Several pairs in copulation may be found here during certain seasons crawling about or hiding in fissures or cracks and under leaves. Eggs, larvae and pupae are also seen in such situations at a depth of half an inch to two inches. The adults repeat copulation several times at intervals of about ten minutes each act lasting for some twenty minutes. The beetles live on algae, decaying vegetable and animal matter and on small animals like live tadpoles, moths and larvae of Syrphid flies.

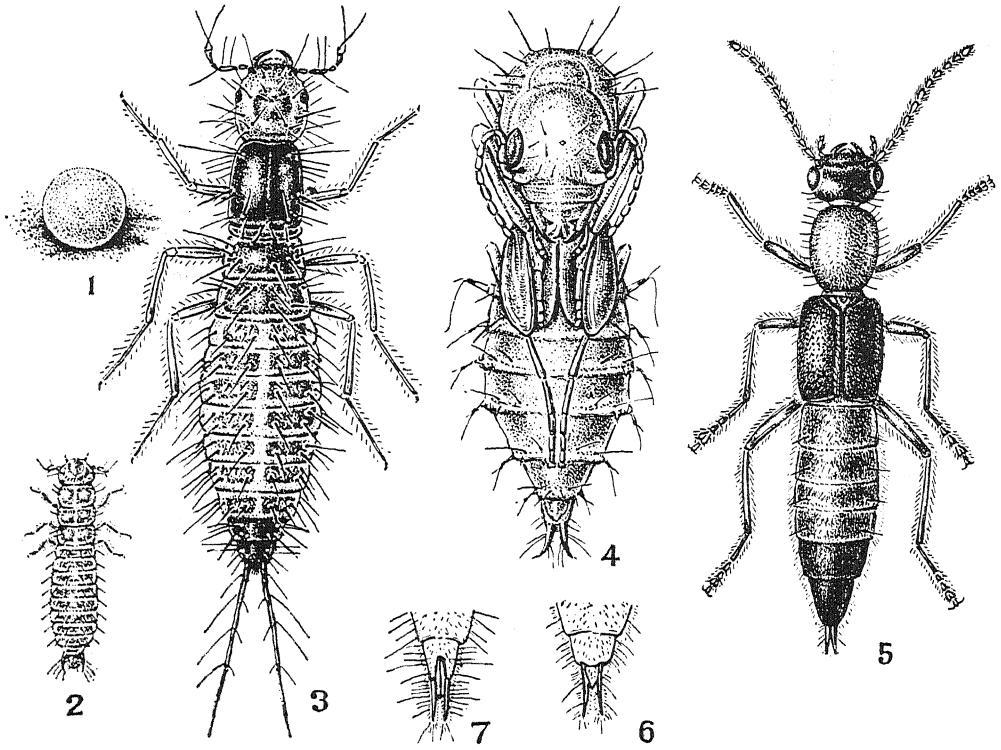
DESCRIPTION OF EARLY STAGES.

The egg. (Plate XIV, fig. 1).—The egg is spherical, light brown and 0·7 mm. in diameter. The egg-shell is leathery and has a slightly pitted surface.

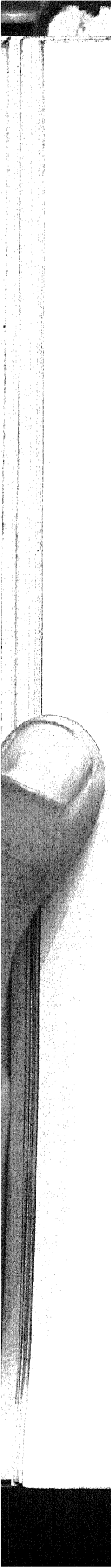
The first stage larva. (Plate XIV, fig. 2).—The larva on hatching is 1·7 mm. long and is shiny slaty-brown in colour. Its greatest width is towards the middle of

PLATE XIV.

PAEDERUS FUSCIPES CURT.



1. Egg ×16. 2. First stage larva ×16. 3. Full-grown larva ×16. 4. Pupa ×16. 5. Adult *Paederus fuscipes* Curt. ×10. 6. Ventral view of posterior extremity of abdomen of female beetle much magnified. 7. Ventral view of posterior extremity of abdomen of male adult (showing excision on sixth sternite) much magnified.



the abdomen which shows ten distinct segments, the ninth carrying a pair of chitinous incurved single-jointed cerci. The short fleshy terminal tenth abdominal segment is seen between the cerci. There are very few setae on the body.

The second stage larva.—After the first moult the larva has the abdomen an orange-brown colour and the cerci on the ninth abdominal segment appear as straight, two-jointed processes. The head is yellow and the thoracic tergites are brownish black.

The third stage (full-grown) larva. (Plate XIV, fig. 3).—The larva when full grown is 4·7 mm. long. There are ten segments in the abdomen. The ninth abdominal segment carries a pair of fine setaceous two-jointed processes. The larva in this stage is grey in colour. The tergites on the eighth and ninth abdominal segments are very dark. The whole body is setaceous. The head is bent slightly downward. The two-jointed cerci are very long. The larva is moderately active.

The pupa. (Plate XIV, fig. 4).—The pupa has no sort of protective covering, is free and 4 mm. long. The head is bent downwards and held close to the thorax. It is white in colour when newly formed, and slowly changes to creamy yellow with an orange tinge in all the abdominal segments except the last two.

Sexual dimorphism in adults.

The male and female adults can be distinguished by examining the ventral surface of the abdomen. In the female (Plate XIV, fig. 6) the sixth sternite is entire, but in the male (Plate XIV, fig. 7) the corresponding sternite has a narrow median excision.

Life-history and larval moults.

The eggs are laid generally in the early morning singly in the soil about an inch below the surface. They hatch in two to three days. Each female beetle lays about six eggs every day for several days. The larva remains in the soil at a depth of half to two inches and is moderately active. The larval period occupies about seven days during which it feeds on decaying matter and also preys on tadpoles and Syrphid larvae. It moults twice. In the third instar the larva becomes full grown, remains quiescent in its usual habitat, moults and changes into pupa. The pupal period extends for three to four days. The life-cycle is, *egg* two to three days, *larva* about seven days and *pupa* three to four days.

Natural enemies.

The larvae and pupae are preyed on by soil mites and Carabid larvae.

Seasonal abundance.

P. fuscipes Curt. becomes active as soon as winter is over. From the beginning of March it multiplies rapidly and adults both free and in copulation, eggs, larvae and pupae occur in abundance and if circumstances are favourable it produces several generations and reaches its greatest numerical strength during the months of May and June. It is chiefly during May and June that the complaint of "spider lick", the vesicular dermatitis of man caused by this beetle becomes widespread and frequent, because of the large number of beetles attracted by artificial lights to enter dwellings.

The increase in numbers of this insect depends, however, on the rainfall. If the rainfall during the months of March, April and May is deficient it is able to multiply to its utmost capacity. Heavy rains during this period will wash away or drown these insects and will also suffocate large numbers of larvae and pupae by making the soil too wet and impervious.

In any case, the onset of the monsoon and subsequent floods from about the end of the month of June, together destroy this species so effectively that very few are seen during the rest of the year. Winter retards its multiplication and it is only in early summer that this insect can again assume pest proportions.

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PLATE XV.

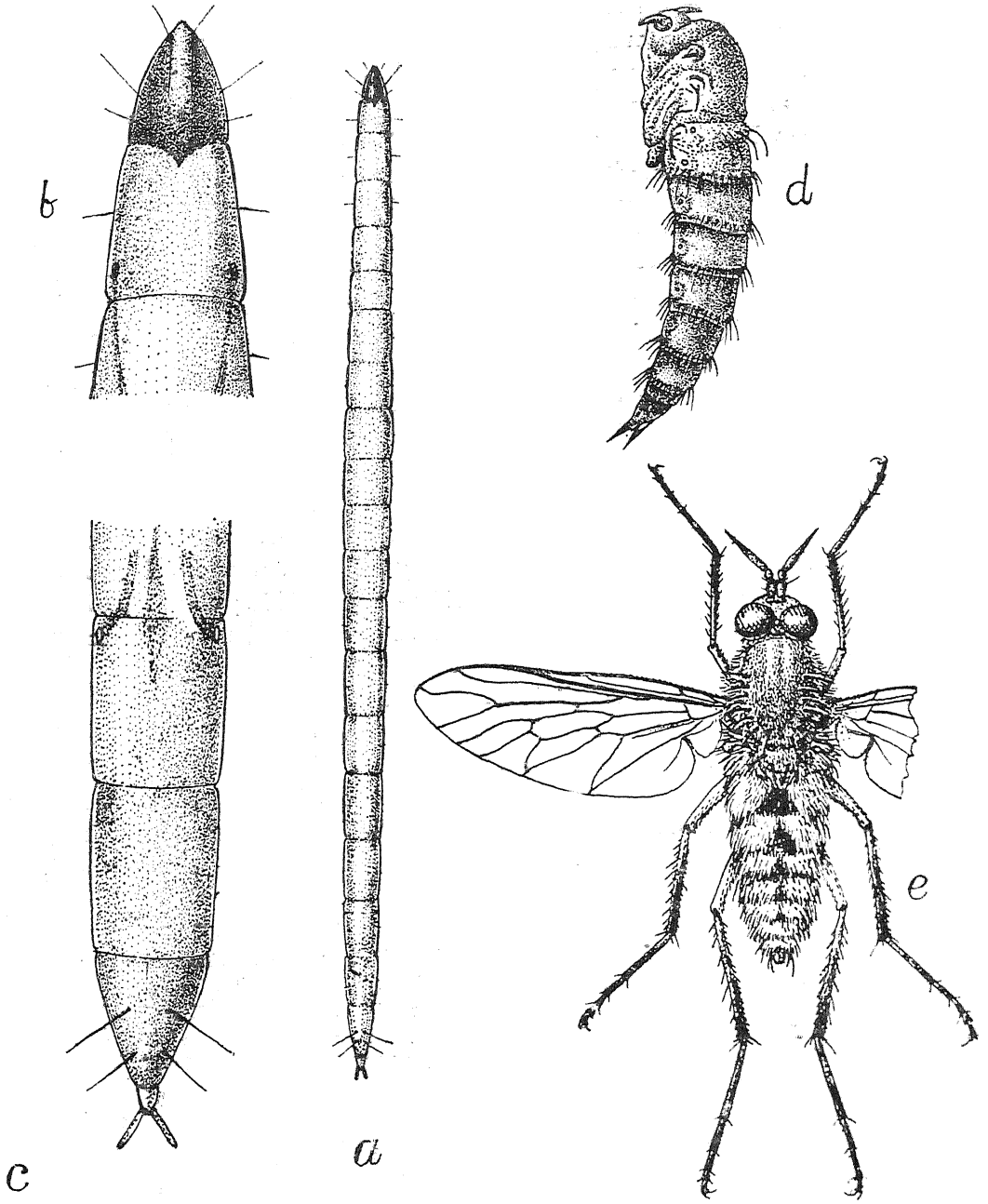


Fig. *a*.—Full-grown larva (×6). Fig. *b*.—Anterior end of the full-grown larva (×24). Fig. *c*.—Posterior end of the full-grown larva (×24). Fig. *d*. Pupa (×6). Fig. *e*.—Imago, male (×6).

A NOTE ON THE LIFE-HISTORY OF *PSILOCEPHALA SEQUA* WALKER (FAM. THEREVIDAE: DIPTERA).

BY

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Assistant to the Imperial Entomologist, Pusa.

(Received for publication on 5th September 1933)

(With Plate XV)

Although considerable taxonomic work has been done on the Family Therevidae in various parts of the world, yet little is known of the early stages, habits and habitats of the various species. The flies resemble superficially the Asilidae but are less bristly and not so active. They are predaceous. The habits of the larvae of only a few species are known. Williston [1908] states that the larvae live in earth or sand and decomposing wood, feeding upon other insects or vegetable matter. Lundbeck [1908] gives the description of the larva of *Thereva* and summarizes the habits of the larvae of some of the European species of the genus. Verrall [1909] confirms Williston regarding the habitats of the larvae and adds that the larvae feed on any vegetable or animal refuse or even affix themselves to living larvae. Felt [1912] describes the early stages of *Psilocephala melampodia* Loew. Malloch [1917] states that the larvae are found in the ground and also in decaying wood and are predaceous, feeding on various insect larvae and are apt to be cannibalistic. Isaac [1925] discovered the larvae of *Phycus brunneus* Wied. feeding on Dermestid larvae among the excreta of bats in crevices and fissures in walls.

Psilocephala sequa WALKER.

The flies have been collected in Pusa (Bihar) from January to July and during October and November. The egg stage is not known. The larvae have been found in fields in Pusa, in March, May and June. They were obtained at a depth of nearly an inch in the soil.

The full-grown larva.—(Plate XV, figs. *a*, *b* and *c*). The fully-matured larva is white in colour with at times a pinkish tinge at places underneath the skin. It is 24 mm. in length and 1·3 mm. in breadth. The small, conical and non-retractile head is dark brown and has three pairs of hairs on the dorsal surface and the sides. The body is smooth and cylindrical tapering towards both ends. It is snake-like in appearance and performs serpentine movements especially when disturbed. It appears to be composed of twenty segments excluding the head. This is because

each one of the first six abdominal segments is divided into two [Malloch, 1917; Isaac, 1925; and Imms, 1930]. The three thoracic segments have each a pair of hairs at about the middle on sides. The penultimate segment bears two hairs on each side. The larva is amphipneustic. The anterior spiracles are situated on the sides of the first thoracic segment near the posterior margin and the posterior spiracles are on the fore end of the sides of the fourth segment from the anal end. The tracheal tubes can be seen on the sides, underneath the skin from the first to the eighth abdominal segment. The anterior and posterior pair of spiracles appear as dots of brown colour on the body of the larva. The last segment is conical bearing a pair of white digitate processes at its end resembling in appearance the prolegs in lepidopterous larvae. The larvae under laboratory conditions showed well-marked cannibalistic tendencies.

Pupa (Plate XV, fig. *d*).—11 mm. long, 2·6 mm. broad; yellowish-white; two well-developed thorns (antennal sheaths) on the sides of the head, a prominent thorn at the base of the wing-sheath on each side and a smaller one occupying a middle position. Abdominal segments, each with a girdle of long spines of yellowish white colour on the posterior margin and in addition to these spines, on the third, fourth and fifth segments smaller spines with black tips, the last segment ending in a pair of well-developed black spines which are red at the tips; the spiracles on thoracic and abdominal segments tubular.

The pupal period varies from seven to eleven days.

The imaginies.—The imago (Plate XV, fig. *e*) escapes from the pupa by a T-shaped slit as in other Brachycera. The male fly can be distinguished from the female by its eyes which are contiguous for some distance. In other respects they are much alike and are of a black colour with dark green eyes and silvery hairs on the face, thorax and the abdominal segments. The tibiae are ochraceous.

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A PRELIMINARY NOTE ON THE EFFECT OF MAIZE-ROOT WASHINGS ON THE FIXATION OF NITROGEN.

BY

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Assistant to the Imperial Agricultural Bacteriologist.

(Received for publication on 9th September 1933)

During the course of investigation in the Bacteriological Section of the Pusa Research Institute, on the nitrogen content of soil, as affected by different systems of cropping, it was observed that in some years the nitrogen content of the soil, in which maize was growing, was slightly higher at the time of its harvest than what it was at the time of sowing. This increase, in the nitrogen content of soil, in some years, suggested that under certain conditions nitrogen fixation might be taking place when the crop was growing in the soil; and as this phenomenon had occurred when maize was growing, it was considered that maize may be responsible for this increase in nitrogen.

Since the methods of determining the nitrogen content of soil in the field are not very accurate, and slight variations of nitrogen content can be regarded as experimental error, it was considered necessary to study the problem further in the laboratory.

In these studies Ashby's medium of the following composition was used:—

Mannite	20·0 grm.
KH_2PO_4	0·2 ,,
$MgSO_4$	0·2 ,,
NaCl	0·2 ,,
$CaSO_4$	0·1 ,,
Water	1000·0 c.c.
$CaCO_3$	0·5 grm. per flask.

KH_2PO_4 was dissolved separately and made just alkaline to phenolphthalein with *N*/14 caustic soda and then added to the solution. 100 c.c. of the above medium were put in Erlenmeyer flasks of 500 c.c. capacity, which were then sterilized at 130°C. for 15 minutes. Each flask was then inoculated with one grm. of air-dry Pusa soil, sieved through half-a-millimeter sieve.

Maize-root washings.

Maize was sown in a plot and after about a week's growth 40 plants were dug out and their roots were washed thoroughly with tap water. They were then

each one of the first six abdominal segments is divided into two [Malloch, 1917; Isaac, 1925; and Imms, 1930]. The three thoracic segments have each a pair of hairs at about the middle on sides. The penultimate segment bears two hairs on each side. The larva is amphipneustic. The anterior spiracles are situated on the sides of the first thoracic segment near the posterior margin and the posterior spiracles are on the fore end of the sides of the fourth segment from the anal end. The tracheal tubes can be seen on the sides, underneath the skin from the first to the eighth abdominal segment. The anterior and posterior pair of spiracles appear as dots of brown colour on the body of the larva. The last segment is conical bearing a pair of white digitate processes at its end resembling in appearance the prolegs in lepidopterous larvae. The larvae under laboratory conditions showed well-marked cannibalistic tendencies.

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Maize-root washings.

Maize was sown in a plot and after about a week's growth 40 plants were dug out and their roots were washed thoroughly with tap water. They were then

allowed to stand in 200 c.c. of distilled water for 24 hours. This water was then filtered through ordinary filter paper and was used as root washings in these experiments to see its effect on the soil flora in Ashby's culture medium.

Five c.c. of this filtrate were added to each of flasks at an interval of three days, the first addition being on the next day after inoculation of the medium with soil. In all, six washings were given to each set of flasks except the controls, to which the same amount of sterile distilled water was added in order to maintain the same consistency of the culture medium. For subsequent washings plants were dug out from the same plot from which they were dug out for the first washings.

Those flasks which received root washings were divided into two sets. The first set in which the action of organisms had to be completely inhibited was treated with 5 c.c. of strong sulphuric acid containing one grm. of salicylic acid dissolved in it. The second set received root washings directly as prepared without any further treatment.

Nitrogen estimation.

At first the flasks which had not received sulphuric and salicylic acid mixture were treated with 5 c.c. of this mixture and then the contents were transferred to 500 c.c. digestion flasks; concentrated and digested as usual with sulphuric acid, copper sulphate and potassium sulphate, for the determination of nitrogen. The digested contents were distilled with caustic soda, ammonia being trapped in $N/14$ sulphuric acid.

Table I.

Mgrms. of nitrogen per 100 c.c. of Ashby's medium containing 2 grms. of mannite.

(Period of observation 13th July 1931 to 3rd August 1931.)

Treatment	Nitrogen in mgrm. (average of quadruplicate flasks)	Nitrogen fixed *minus* the nitrogen in the media	Nitrogen fixed by treatment over the control
1. (Medium + soil + sulphuric and salicylic acid mixture.)	0·55	..	..
2. (As above with root washings) . . .	0·70	..	..
3. Control (medium + soil) . . .	13·675	13·125	..
4. (Medium + soil + root washings) . .	14·675	13·975	0·850

The figures, shown in the above table, distinctly show that the maize-root washings stimulated the soil organisms to fix more nitrogen in the medium ; the actual extra amount of nitrogen fixed being 0·85 mgrm. per 100 c.c. of the medium over the nitrogen fixed in the medium without root washings.

The organisms added with root washings in this experiment were not excluded and thus the extra amount of nitrogen fixed may be attributed, either to the stimulating effect of root washings or to the activities of the new organisms added along with the washings. In order to prevent the access of such organisms in the culture flasks, a second series was started in which the washings were sterilized (*a*) by passing through a filter candle, (*b*) by steam at 120°C. for 30 minutes. The results of this series are shown in Table II.

TABLE II.

Mgrms. of nitrogen per 100 c.c. of Ashby's medium containing 2 grms. of mannite. Quadruplicate flasks were used in the 2nd and the 3rd series and 6 flasks in the last series.

Treatment	2nd series 29-9-31 to 22-10-31			3rd series 6-7-32 to 27-7-32			4th series 9-9-32 to 30-9-32		
	Total nitrogen	Nitrogen fixed *minus* the nitrogen in the media	Nitrogen fixed by treatment over the control	Total nitrogen	Nitrogen fixed *minus* the nitrogen in the media	Nitrogen fixed by treatment over the control	Total nitrogen	Nitrogen fixed *minus* the nitrogen in the media	Nitrogen fixed by treatment over the control
1. Medium + soil + sulphuric and salicylic acid mixture.	0·85	..	..	0·7	..	..	0·7	..	..
2. As above with root washings .	1·25	..	..	1·0	..	..	0·95	..	..
3. Control (medium + soil) . .	14·35	13·5	..	13·6	12·9	..	14·52	13·82	..
4. Medium + soil + root washings.	15·95	14·7	1·2	14·475	14·475	1·575	15·77	14·82	1·0
5. As No. 4 but washings filtered through filter candle.	17·85	16·6	3·1	15·925	14·925	2·025	16·92	15·97	2·15
6. As No. 4 but washings sterilized at 120°C. for 30 minutes.	15·85	14·6	1·1	15·05	14·05	1·15	16·10	15·15	1·33
7. As No. 4 but washings sterilized at 100°C. for 3 consecutive days.	..	..	..	..	..	..	15·83	14·88	1·06

From the figures presented in the table, it will be seen that they confirm the previous observation of the stimulating effect of the maize-root washings on nitrogen fixing organisms present in the soil inoculum. Further they also show that the root washings sterilized by passing through filter candle are more effective than either the unsterilized washings or the washings sterilized at 120°C. for 30 minutes.

As the season had advanced by this time, normal growth of the maize seedling was not possible and hence further experiments to confirm the above results were carried out in the rains of 1932, the results of which are given in the last two series of the same table.

The figures presented in these two series are the averages of four and six flasks respectively and they confirm the results obtained in 1931.

In the last series in which six sets of flasks were used, the amount of nitrogen fixed by unsterilized root washings was one mgrm. over the control and the candle filtered washings increased it to 2·15 mgrm. (15·56 per cent. over the control). When steam at 120°C. for 30 minutes or at 100°C. for 3 consecutive days was used for sterilization the increase came to 1·33 mgrm. and 1·06 mgrm. respectively.

Summing up the results, we are led to conclude, that maize roots secrete some products which stimulate nitrogen fixing organisms present in the soil inoculum to fix more nitrogen in Ashby's mannite solution. This may explain why the amount of nitrogen in the soil, in which maize is grown, is found higher, in some seasons, at the time of its harvest than the amount present at the time of its sowing.

The nature of the substance or substances thus secreted by maize, helping the nitrogen fixing organisms in the soil and the conditions under which such substances are secreted so as to stimulate fixation of nitrogen under field conditions, are problems yet to be investigated.

Whether any other cereals or non-leguminous plants secrete a similar substance is also a question worth investigating.

FIRST YEAR (1932-33) RIPENING TESTS WITH SUGAR-CANE × SORGHUM CROSSES.

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This note is a brief statement of chemical studies on the ripening of sugar-cane × sorghum hybrids, carried out during the season 1932-33.

The hybrids are seven in number—Co. 351, Co. 352, Co. 353, Co. 354, Co. 355, Co. 356 and Co. 357—and are the new creations of Rao Bahadur T. S. Venkatraman, the Government Sugarcane Expert, Coimbatore. Having *Andropogon Sorghum* as one of the parents, these hybrids are expected to come to maturity in six months from the time of planting. Actually, the Government Sugarcane Expert, Coimbatore, found them to come to maturity in six months, both as seedlings and sett plants and analysing better than ordinary sugarcane [Venkatraman, 1932]. For ready reference the analytical data given by Venkatraman are reproduced below :—

Juice analyses of sugarcane-sorghum hybrids at 6 months.

Seedling No.	Brix	Sucrose	Glucose	Coefficient of purity
	Per cent.	Per cent.	Per cent.	
Co. 351	20·42	18·53	0·17	90·8
Co. 352	19·31	17·33	0·26	89·6
Co. 353	19·01	16·75	0·42	88·1
Co. 354	18·38	16·18	0·41	88·0
Co. 355	17·71	15·22	0·58	85·9
Co. 356	18·51	16·11	0·50	87·0
Co. 357	20·15	18·00	0·18	89·3

For the purpose of ascertaining the behaviour of these seedlings in the different tracts in the Madras Presidency, the material kindly supplied by the Government Sugarcane Expert was grown in the season 1932-33 on the several Agricultural Stations, chief of them being Anakapalle, Palur, Maruteru, Aduturai and Coimbatore. The authors are indebted to the Superintendents of these stations for the supply of the material for chemical analysis, which forms the basis for the work reported in this paper.

Tables showing amounts of monthly rainfall with their departures from normals, for the several stations, and temperature and humidity data either for the experiment stations or for places in the vicinity of the research stations are appended (Tables I and II).

Systematic periodical analyses of the crops at different stages of their growth were conducted, only from the month of November 1932, although, at Anakapalle, it was possible to commence in September. At Aduturai the first series of analyses were done only in the month of January 1933 when the canes were nearly ten months old. At Maruteru the analyses commenced towards the close of November when the seedlings were 270 days old.

The material for the tests was very small. It was not possible to plant more than one or two lines under each variety. The number of canes per sample rarely exceeded twenty and there were instances in which only four and five canes could be had for crushing. This fact should be remembered in examining the figures for chemical analyses and they should not be taken as absolute values for rigorous comparisons. For the same reason tonnage and available sugar per acre are not calculated. Nevertheless, the available data is adequate to show the trend of the progress of ripening of the new creations and to permit preliminary conclusions in regard to the performance of the sorghum hybrids at the several agricultural stations when planted at different periods of the year.

Performance of the hybrids planted in March—April 1932.

At Anakapalle the hybrids were planted in different fields and under different treatments. These will be discussed later in their appropriate places. The hybrids planted in Field No. 33 attained their maximum sugar content earliest. The figures for this plot are therefore taken for the study of the march of ripening as judged by the chemical analyses of the juices. The results of periodical analyses of sorghum hybrids in Field No. 33 Anakapalle are presented in the form of a table (Table III).

When planted in March or April, a purity of 85 was attained in about eight months, but the value for sucrose was 15 per cent. on the weight of juice. In about

300 days the sucrose content went up as high as 19 per cent. and the purity approached 90. Sustained and continued decrease in the glucose content commenced more definitely, only when the crop was about 250 days old. The comparative performance of crops of similar ages at the different experiment stations is brought out in the tabular statements. Comparable data for this period are available only with reference to the two stations—Anakapalle and Palur, for the March-April planted crop and the tables that have been prepared with reference to their ages—after 195 days (Table IV), 230 days (Table V), and 275 days (Table VI), will indicate the behaviour of these seedlings in two distant places as Anakapalle and Palur.

It will be noticed that two points emerge from the above data. The first is that under different environmental conditions the hybrids analysed poorly and were not ripe in seven months and continued to improve after that period ; the second is that the crop at Palur analysed higher than that at Anakapalle.

When the crop was 230 days old, the coefficient of purity went up to 85 and this was in evidence more distinctly at Palur.

After a growth of 300 days, conditions at Anakapalle showed a decided improvement. The sucrose content increased from 15 per cent. to 19 per cent. while the value for coefficient of purity rose correspondingly to 90. A marked diminution in glucose was visible only after the crop grew for 250 days at Anakapalle. At Palur, the maximum sucrose content was registered after 230 days and remained steady at that point but the value for the coefficient of purity showed a tendency to rise. Glucose showed a tendency to diminish even from 193 days and continued to do so till 270 days. No analyses could be done beyond this period as no crop was available.

From a study of the progress of ripening of the March-April planted crop it would appear that, both at Anakapalle and Palur, Co. 357 showed a tendency to ripen in about eight months, although this variety took longer for its maximum performance. Co. 355 was the latest, taking as long as eleven months. At Maruteru the first set of analyses could be done only towards the end of November when the canes were nearly nine months old. At this time only Co. 352 had shown signs of maturity while the other two varieties, Co. 353 and Co. 355, were still immature. These two varieties analysed very well when they had 340 days' growth, giving a sucrose content of 19 per cent. and a purity coefficient of 91. Co. 351 also showed a tendency towards early maturity but not as definitely as Co. 357 did at Anakapalle or Palur.

Thus, these hybrids, with the exception of Co. 351 and Co. 357, required nine months to mature when planted in March-April season. When kept on the field beyond this time they were, however, capable of further improvement register-

ing such high values as 20 to 21 per cent. brix and 19 to 20 per cent. sucrose on the weight of juice. The average weight of cane at Anakapalle was slightly more than 2·5 pounds but this was obtained when the crop has had its ten months of growth. At Palur the cane weight was relatively poorer.

Performance of late-planted sorghum hybrids.

In Southern India sugarcane continues to grow till the monsoon rains cease and comparatively cooler weather sets in about the middle of November. After this period growth either ceases or considerably slows down and the crop begins to mature. The observed delayed maturity of the March-April planted crop might be due to the long period of growth. The effect of planting in the month of June just before the commencement of South-West monsoon showers and in the month of September just after the cessation of the monsoon period, was therefore under study at all the experiment stations.

June-planted crop.

At Anakapalle all the seven varieties were under trial but at the other stations only the three varieties—Co. 352, Co. 353 and Co. 355—were grown. It will be sufficient to follow the course of analyses of the three varieties at the several stations and for this purpose comparative statements of analyses after 200-210 days' growth and after 235-245 days' growth are appended (Tables VII and VIII).

It will be noticed that when planted in the month of June the crop comes to maturity in about 240 days but the richness of the juice does not compare favourably with that of the March-April planted crop. The June-planted crop had a shorter period of growth and naturally yielded a smaller weight of cane as judged by the average weight of cane drawn for the sample. Further keeping of the crop on the field after a growth of 240 days, does appear to show a further improvement in the values for brix and sucrose. This will be seen from the appended table (Table IX) containing the statement of figures showing the gain or loss over the previous analyses done at the time when the crop has had a growth of 240 or 245 days.

As stated earlier in the report it must be mentioned here that no significance should, however, be attached to these values in regard to the magnitude of their appreciation or depreciation. They can be interpreted only as showing a tendency for further improvement and that the maximum capacity of these seedlings was not attained in 240 days' time.

September 1932 planting.

At all the stations the September-planted crops analysed poorly. For the sake of brevity and economy in space, detailed tabular statements of analyses are not

presented but only the magnitude and the range of values for the glucose, sucrose and coefficient of purity at the different stations are given (Table X).

At Anakapalle another round of analyses at the age of 264 days showed a further decline in quality. At Palur analyses could not be done before 242 days as the cane growth was so poor that only three or four internodes were above ground before this. At Aduturai the growth was so poor that it was not possible to carry out an analysis even in the month of May.

From a consideration of the results of analyses of the crops planted at different months of the year it seems permissible to make the general observation that irrespective of the time of planting, the crop ceases vegetative growth with the cessation of rains and, with the onset of cold weather, enters into another phase of activity in which solids and sucrose are elaborated, and finally the crop is brought to what is called ripe or mature stage. It would also appear from a study of the figures of chemical analysis that the richness and quality of the juice is associated with a certain optimum amount of vegetative growth.

Season, that indefinite factor, has a potent influence on the growth and maturity of the sugarcane crop. This is reflected in the composition and quality of the juice. As an instance in point, may be mentioned the analyses of the juices of sorghum hybrids planted in September 1931 and analysed in April 1932 and those planted in September 1932 and analysed in April 1933 at Anakapalle and these are presented in a tabular form (Table XI) in which the maximum and minimum values for the seven varieties are given.

It is therefore, necessary, in order to form a correct picture of the march of ripening of the sorghum hybrids to have a knowledge of the comparative values for some of the other varieties of sugarcane at the respective stations. A comparative tabular statement giving the results of analyses of cane varieties planted along side the sorghum hybrids is given (Table XII). It will be noticed that several other varieties of sugarcanes have also analysed similarly.

Effect of manurial and other conditions on the maturity of sorghum hybrids.

At Anakapalle some intensive work was done by growing the hybrids in different soils and under different cultural and manurial treatments. The following were the fields and the treatments given.

Field No. 33 :—Wet land—10 cart loads farmyard manure, 250 lbs. ammonium sulphate, 225 lbs. super. and 2,000 lbs. Wild indigo. Crop was planted on 11th April 1932.

Field No. 24-A :—Wet land—10 cart loads farmyard manure, 125 lbs. ammonium sulphate, 225 lbs. super. Crop was planted on 14th April 1932.

In Table XIII the analytical values showing the march of ripening are given for only two varieties, Co. 352 and Co. 353, which are selected as representing the type. It will be clear that manurial treatment did not prolong the period of maturity and that the April-planted crop requires about ten months for its maximum output.

Summary.

1. The results of first year's chemical analyses of juices conducted in 1932-33 at the several agricultural stations to test the maturity period of certain sugarcane-sorghum hybrids are reported.

2. The early or March-planted crop matured in ten months. The June-planted crop matured in 8 to 9 months, but the quality of the crop and the juice were poorer than that of the March-April planted crop. The September-planted cane gave juices of still poorer quality. With the exception of Co. 357 which attained a purity value round about 85 in 8 months, the other varieties registered very low purity even long after this period, thus indicating that the period of maturity of the September-planted crop was not definite.

3. Compared with other sugarcane varities, the sorghum hybrids tend to come to maturity a little earlier, but as in the case of sugarcane, the ripening of the hybrids is coincident with cooler months and lower humidity.

4. The juices of the sorghum hybrids at the point of their maximum efficiency were decidedly richer than those of other Coimbatore canes. Whether this is characteristic of the sorghum hybrids can be judged only after further experience.

5. From the results presented, it would appear that certain of these sorghum hybrids are capable of attaining a purity of 85 and over in about 200 to 220 days from the time of planting and tend to improve in quality for nearly 100 days afterwards. This, if confirmed by subsequent experiences, would be a distinct advantage in extending the milling season.

REFERENCE.

Venkatraman, T. S. (1932). *Ind. J. Agric. Sci.* 2, 22.

TABLE I.

Amounts of monthly rainfall, in inches, and their departures from normal for the year 1932-33.

Month	Anakapalle		Maruteru		Palur		Aduturai		Coimbatore	
	Actual	Departure from normal	Actual	Departure from normal	Actual	Departure from normal	Actual	Departure from normal	Actual	Departure from normal
1932.										
March .	0	..	..	..	..	—0·1	..	..	..	—0·8
April . .	2·59	1·1	..	..	3·6	2·6	..	..	2·8	1·4
May . .	0·80	—1·9	3·4	2·9	1·8	0·7	3·6	1·1	6·1	3·9
June . .	3·1	—2·9	2·5	—1·5	0·4	—0·6	0·6	—0·9	0·9	—1·0
July . .	8·0	2·8	10·3	3·6	..	..	1·9	0·4	1·3	1·1
August .	2·0	—1·6	6·8	1·9	4·5	—1·2	2·8	—0·4	2·8	1·6
September .	7·2	—0·6	3·0	—3·8	5·8	3·8	2·0	—1·3	0·4	—1·6
October .	2·4	—7·2	4·8	—8·3	9·6	6·9	11·6	2·9	6·6	0·7
November .	8·5	4·2	6·4	2·8	11·7	8·1	18·1	8·4	7·2	2·5
December .	..	—0·3	..	—0·2	6·1	2·3	8·7	—0·6	..	..
1933.										
January .	..	..	..	..	..	..	..	—5·6	..	—0·9
February .	..	—1·9	..	..	0·1	—0·4	0·8	0·6	..	—0·5
March .	0·3	0·2	0·05	0·04	12·4	8·3	1·8	0·5	..	..

TABLE II.

Maximum and minimum temperatures and humidity with their deviations from normal at Coimbatore, Vizagapatam in the vicinity of Anakapalle and Cuddalore in the vicinity of Palur for 1932-33.

Month	Coimbatore						Vizagapatam						Cuddalore					
	Maximum		Minimum		Humidity		Maximum		Minimum		Humidity		Maximum		Minimum		Humidity	
	Mean	Deviation	Mean	Deviation	Mean	Deviation	Mean	Deviation	Mean	Deviation	Mean	Deviation	Mean	Deviation	Mean	Deviation	Mean	Deviation
1932																		
February .	88·8	—2·7	67·4	1·4	72	—8	82·1	—1·7	69·7	—1·4	85	12	85·4	—0·2	70·5	0·7	84	—2
March .	92·2	—3·9	70·9	1·0	67	—11	85·2	—2·1	71·6	—3·1	73	0	86·9	—2·2	71·8	—0·7	78	—4
April . .	93·7	—3·6	73·7	0·2	75	—4	88·4	—1·3	77·8	—0·5	73	2	91·0	—1·8	77·5	..	79	1
May . .	89·4	—5·4	73·1	0·4	78	—2	89·2	—2·8	81·0	0·2	77	6	93·5	—4·9	79·7	—0·6	70	..
June . .	86·8	—2·5	72·2	0·4	76	—4	91·9	0·7	81·3	1·1	79	5	100·0	1·3	80·9	0·8	58	—9
July . .	84·8	—2·7	71·8	0·9	76	—5	87·6	—1·4	78·5	—0·1	87	9	96·8	1·1	79·6	1·3	66	—7
August .	87·0	—1·1	72·2	1·3	80	—3	87·9	—0·9	79·1	0·9	79	—1	92·0	—2·0	78·0	1·0	79	2
September .	86·1	—3·0	71·5	0·7	76	—7	89·2	0·8	79·1	1·0	79	..	95·0	2·8	77·7	1·3	75	—4
October .	85·6	—2·4	71·7	1·1	84	..	87·9	—0·1	77·5	1·1	75	1	87·2	—1·4	76·2	1·3	87	3
November .	83·7	—2·1	70·1	1·2	82	—1	83·6	—0·7	74·8	2·1	73	8	85·1	0·3	74·1	1·6	86	—1
December .	83·2	—1·5	66·1	0·4	74	—8	81·1	0·3	66·1	—2·0	80	17	82·3	—0·5	71·0	1·1	86	..
1933																		
January .	85·3	—1·1	65·8	1·5	71	—11	80·6	—0·2	66·5	—1·4	85	13	83·2	0·1	70·8	2·5	83	—4
February .	83·0	—2·0	70·5	—2·5	80	7	84·6	—2·4	69·6	—1·4	83	—1	90·0	—4·0	68·0	..	68	—10
March .	86·0	..	73·0	..	76	2	86·0	—2·0	73·0	1·0	83	..	93·6	—1·4	71·0	3·0	68	—9

NOTE.—Vizagapatam and Cuddalore are taken to correspond to Anakapalle and Palur.

TABLE III.

Anakapalle—April-planted crop. Date of planting 11th April 1932—Field No. 33, wet land, irrigated. 100 lbs. nitrogen.

Variety	Age of the crop (days)	Average weight of a cane (lbs.)	Brix (Per cent.)	Sucrose (Per cent.)	Glucose (Per cent.)	Co-efficient of purity	Top/bottom ratio	Date of analysis	Remarks
Co. 351 . .	158	1·80	10·22	5·77	2·23	58·5	0·75	15th Sept. 1932	
	197	2·08	14·77	10·78	1·81	73·0	0·91	24th Oct. ,,	
	227	1·83	17·47	14·61	1·00	86·6	0·94	28th Nov. ,,	
	253	2·50	18·78	16·21	0·66	86·3	1·00	21st Dec. ,,	
	293	2·40	21·02	18·50	0·52	88·0	1·05	30th Jan. 1933	
	335	2·70	21·12	18·72	0·64	88·6	1·03	13th March ,,	
Co. 352 . .	158	1·90	11·32	7·05	2·34	62·3	0·82	15th Sept. 1932	
	197	2·00	11·02	6·36	2·01	57·7	0·88	24th Oct. ,,	
	227	2·50	14·18	11·19	1·66	78·9	0·96	28th Nov. ,,	
	253	2·07	14·18	11·05	1·18	77·9	0·96	21st Dec. ,,	
	293	2·93	19·42	17·39	0·29	89·5	1·10	30th Jan. 1933	
	335	2·46	19·12	16·36	0·45	85·6	1·10	13th March ,,	
Co. 353 . .	158	2·10	10·52	6·42	2·44	61·3	0·74	15th Sept. 1932	
	197	2·53	11·72	6·54	2·34	55·8	0·83	24th Oct. ,,	
	227	3·13	15·02	11·99	1·59	79·8	0·97	28th Nov. ,,	
	253	2·75	16·48	13·69	0·94	83·1	1·06	21st Dec. ,,	
	293	3·34	18·99	16·27	0·69	85·7	1·16	30th Jan. 1933	
	335	2·98	18·19	15·36	0·77	84·5	1·25	13th March ,,	
Co. 354 . .	158	1·00	11·52	7·74	2·01	67·2	0·71	15th Sept. 1932	
	197	1·10	13·85	9·40	1·55	67·9	0·82	24th Oct. ,,	
	227	1·40	15·42	12·76	1·66	82·7	0·89	28th Nov. ,,	
	253	1·35	16·86	13·92	1·04	82·5	0·93	21st Dec. ,,	
	293	1·74	19·02	16·96	0·50	89·2	0·96	30th Jan. 1933	
	335	1·54	18·99	16·23	0·71	85·5	1·05	13th March ,,	

Co. 355 . .	158	1·80	10·72	6·86	1·92	64·0	0·74	15th Sept. 1932
	197	1·70	13·35	8·18	1·20	61·3	0·85	24th Oct. ,,
	227	2·58	15·22	12·03	1·60	79·0	0·84	28th Nov. ,,
	253	2·63	16·76	13·68	1·51	81·6	0·90	21st Dec. ,,
	293	2·79	17·00	13·92	1·58	81·9	1·02	30th Jan. 1933
	335	2·95	19·39	16·56	0·83	85·4	0·98	13th March ,,
Co. 356 . .	158	2·45	10·52	5·97	2·35	56·8	0·75	15th Sept. 1932
	197	2·10	12·42	7·96	1·10	64·1	0·86	24th Oct. ,,
	227	2·68	15·56	12·56	1·66	80·7	0·99	28th Nov. ,,
	253	2·91	17·24	13·56	1·17	78·7	1·02	21st Dec. ,,
	293	2·97	20·49	18·21	0·73	88·9	1·02	30th Jan. 1933
	335	2·60	18·18	14·58	0·59	80·2	0·98	13th March ,,
Co. 357 . .	158	2·03	10·62	6·77	1·93	63·8	0·86	15th Sep. 1932
	197	2·00	13·65	8·57	1·84	62·8	0·96	24th Oct. ,,
	227	2·00	17·03	15·05	0·71	88·4	1·04	28th Nov. ,,
	253	3·07	18·29	15·95	0·36	87·2	1·09	21st Dec. ,,
	293	3·20	20·99	18·74	0·34	89·3	1·09	30th Jan. 1933
	335	2·47	21·39	19·20	0·41	89·8	1·19	13th March ,,

TABLE IV.

March-April planted canes.

Variety	Place	Date of analysis	Age in days	Brix	Sucrose	Glucose	Purity	T/B ratio	Average weight of a cane (lbs.)
Co. 351 .	Anakapalle . .	24th October 1932 .	197	14·77	10·78	1·81	73·0	0·91	2·08
	Palur . . .	17th October 1932 .	193	17·24	14·11	1·56	81·3	0·97	1·71
Co. 352 .	Anakapalle . .	24th October 1932 .	197	11·02	6·36	2·01	57·7	0·88	2·00
	Palur . . .	17th October 1932 .	193	16·43	13·52	1·43	82·3	0·92	2·08
Co. 353 .	Anakapalle . .	24th October 1932 .	197	11·72	6·54	2·34	55·8	0·83	2·53
	Palur . . .	17th October 1932 .	193	15·83	12·50	1·80	79·0	0·96	2·33
Co. 355 .	Anakapalle . .	24th October 1932 .	197	13·35	8·18	1·20	61·3	0·85	1·70
	Palur . . .	17th October 1932 .	193	15·03	11·27	2·38	75·0	0·89	1·83
Co. 356 .	Anakapalle . .	24th October 1932 .	197	12·42	7·96	1·10	64·1	0·86	2·10
	Palur . . .	17th October 1932 .	193	16·33	12·22	2·27	74·8	0·92	2·33
Co. 357 .	Anakapalle . .	24th October 1932 .	197	13·65	8·57	1·04	62·8	0·96	2·00
	Palur . . .	17th October 1932 .	193	18·24	15·71	1·19	86·1	0·96	2·29

TABLE V.

March-April planted canes.

Variety	Place	Date of analysis	Age in days	Average weight of a cane in lbs.	Brix	Sucrose	Glucose	Purity	T/B ratio
Co. 351	Anakapalle	25th November 1932	227	1·83	17·47	14·61	1·00	86·6	0·94
	Palur	29th November 1932	232	1·90	20·65	18·13	0·98	87·8	1·03
Co. 352	Anakapalle	25th November 1932	227	2·50	14·18	11·19	1·66	78·9	0·96
	Palur	29th November 1932	232	1·90	17·74	15·39	0·83	86·8	0·99
Co. 353	Anakapalle	25th November 1932	227	3·13	15·02	11·99	1·59	79·8	0·97
	Palur	29th November 1932	232	2·50	17·34	14·81	1·00	85·4	1·01
Co. 355	Anakapalle	25th November 1932	227	2·58	15·22	12·03	1·60	79·0	0·84
	Palur	29th November 1932	232	2·00	19·45	17·12	0·69	88·0	0·90
Co. 356	Anakapalle	25th November 1932	227	2·68	15·56	12·56	1·66	80·7	0·99
	Palur	29th November 1932	232	2·10	19·34	16·52	1·02	85·4	0·96
Co. 357	Anakapalle	25th November 1932	227	2·00	17·03	15·05	0·71	88·4	1·04
	Palur	29th November 1932	232	2·00	20·45	18·16	0·54	88·8	1·04

TABLE VI.

March-April planted canes.

Variety	Place	Date of analysis	Age in days	Weight of a cane in lbs. (average)	Brix	Sucrose	Glucose	Purity	T/B ratio
Co. 351 .	Anakapalle . .	30th January 1933 .	293	2·49	21·02	18·50	0·52	88·0	1·05
	Palur . . .	8th January 1933 .	275	1·54	18·45	17·09	0·42	92·6	1·01
Co. 352 .	Anakapalle . .	30th January 1933 .	293	2·93	19·42	17·39	0·29	89·5	1·10
	Palur . . .	....	275	1·67	17·95	16·56	0·38	92·3	1·00
	Maruteru . .	....	270	2·69	16·67	14·62	0·53	87·7	0·98
Co. 353 .	Anakapalle . .	30th January 1933 .	293	3·34	18·99	16·27	0·69	85·7	1·16
	Palur . . .	8th January 1933 .	275	1·56	18·34	16·59	0·67	90·5	1·04
	Maruteru . .	....	270	3·13	16·37	13·26	0·67	81·0	0·89
Co. 355 .	Anakapalle . .	30th January 1933 .	293	2·79	17·00	13·92	1·58	81·9	1·02
	Palur . . .	8th January 1933 .	275	1·88	18·28	16·97	0·31	92·8	0·96
	Maruteru . .	....	270	2·58	14·16	10·61	0·94	74·9	0·88
Co. 356 .	Anakapalle . .	30th January 1933 .	293	2·97	20·49	18·21	0·73	88·9	1·02
	Palur . . .	8th January 1933 .	275	2·10	19·21	17·84	0·60	92·9	0·99
Co. 357 .	Anakapalle . .	30th January 1933 .	293	3·20	20·99	18·74	0·34	89·3	1·09
	Palur . . .	8th January 1933 .	275	1·88	20·52	19·28	0·26	94·0	1·00

TABLE VII.

June-planted canes 200-210 days old.

Variety	Place	Date of analysis	Age in days	Average weight of a cane in lbs.	Brix	Sucrose	Glucose	Purity	T/B ratio
Co. 352	Anakapalle	26th December 1932	203	1·30	15·60	11·54	1·18	74·0	0·91
	Palur	8th January 1933	208	1·33	17·27	14·27	1·25	82·6	0·99
	Maruteru	19th January 1933	212	1·83	16·57	12·48	2·77	75·3	0·91
	Aduturai	11th January 1933	208	1·50	19·30	17·29	0·23	89·6	0·96
	Central Farm	10th January 1933	208	1·00	17·36	14·97	0·66	86·3	0·85
Co. 353	Anakapalle	26th December 1932	203	1·40	12·82	8·44	2·51	65·8	0·83
	Palur	8th January 1933	208	1·33	17·57	14·60	1·35	83·1	0·86
	Maruteru	19th January 1933	212	2·67	15·57	12·29	1·59	78·9	1·01
	Aduturai	11th January 1933	208	1·52	18·57	16·33	0·46	88·9	0·95
	Central Farm	10th January 1933	208	1·02	16·66	13·55	0·89	81·3	0·84
Co. 355	Anakapalle	26th December 1932	203	1·58	11·85	6·98	2·39	58·9	0·76
	Palur	8th January 1933	208	1·21	16·19	12·95	1·79	80·0	0·86
	Maruteru	19th January 1933	212	1·67	17·48	14·53	1·28	83·2	1·02
	Aduturai	11th January 1933	208	0·80	18·27	16·43	0·38	89·9	0·95
	Central Farm	10th January 1933	208	1·06	17·46	14·91	0·59	85·4	0·82

TABLE VIII.

June-planted canes 235-245 days old.

Variety	Place	Date of analysis	Age in days	Average weight of a cane in lbs.	Brix	Sucrose	Glucose	Purity	T/B ratio
Co. 352	Anakapalle	6th February 1933	247	1·18	20·82	18·04	0·34	86·6	1·00
	Palur	13th February 1933	244	1·63	20·15	18·30	0·40	90·8	1·02
	Maruteru	16th February 1933	237	2·35	19·55	16·33	1·37	83·5	0·95
	Adutu rai	10th February 1933	238	1·08	17·84	15·42	0·26	86·4	0·95
	Central Farm	8th February 1933	236	0·96	19·78	17·11	0·16	86·5	0·93
Co. 353	Anakapalle	6th February 1933	247	1·61	17·50	14·38	1·15	82·2	0·92
	Palur	13th February 1933	244	1·38	21·02	19·42	0·24	92·4	0·96
	Maruteru	16th February 1933	237	2·55	18·84	15·90	1·10	84·4	1·08
	Aduturai	10th February 1933	238	1·00	19·98	17·75	0·14	88·8	0·95
	Central Farm	8th February 1933	236	1·40	19·82	17·02	0·20	89·5	0·98
Co. 355	Anakapalle	6th February 1933	247	1·93	15·10	10·77	1·39	71·3	0·78
	Palur	13th February 1933	244	1·29	19·07	16·91	0·81	88·7	0·90
	Maruteru	16th February 1933	237	2·10	19·85	17·61	0·53	88·7	1·00
	Aduturai	10th February 1933	238	0·33	15·53	13·00	0·36	83·7	0·97
	Central Farm	8th February 1933	236	1·23	19·78	16·90	0·16	85·4	0·92

TABLE IX.

Increase or decrease in the values for brix and sucrose over those of the previous analyses done when 240 or 245 days old.

Station and age of crop in days	Age of the crop at the time of the previous analysis	Co. 352		Co. 353		Co. 355	
		Increase or decrease in		Increase or decrease in		Increase or decrease in	
		Brix	Sucrose	Brix	Sucrose	Brix	Sucrose
Anakapalle 285	247	1·77	1·93	2·18	3·20	0·98	1·99
Palur 275	244	1·71	1·67	0·54	0·49	—0·13	—0·32
Maruteru	No analysis beyond 247 days						
Aduturai 270	238	3·05	3·36	—1·11	—1·31	—2·74	—3·43
Central Farm Coimbatore 328	236	2·42	2·14	3·16	3·47	2·32	1·99
		0·49	0·72	0·25	0·29	0·61	1·04

TABLE X.

Giving the magnitude and the range of values for sucrose, glucose and purity.

Station and age of crop in days	Sucrose per cent.	Glucose per cent.	Coefficient of purity per cent.
Anakapalle 196	8 to 14	2·3 to 1·0	64 to 80
Do. 228	10 to 14	2·0 to 1·0	70 to 85
Do. 253	7 to 14	1·9 to 0·4	59 to 84
Palur 242	7 to 12·5	3·3 to 1·9	68 to 82

NOTE.—The higher values for purity and sucrose refer to Co. 357.

TABLE XI.

Maximum and minimum values for sucrose, glucose and purity for all the 7 varieties at Anakapalle.

Date of planting	Date of analysis	Age in days	Sucrose		Glucose		Purity	
			Max.	Min.	Max.	Min.	Max.	Min.
Sep. 1931 . .	Apr. 1932 .	221	18·0	12·0	0·82	0·31	89	77
Sep. 1932 . .	Apr. 1933 .	228	15·0	9·0	2·10	1·00	86	72

TABLE XII.

Comparison of sorghum hybrids with other canes. Anakapalle—March-April 1932 plantings.

Description of variety	Age in days	Coefficient of purity per cent.	Sucrose per cent.	Glucose per cent.
Other sugarcane varieties—				
M. A. 21	227	84·0	14·4	1·1
Co. 213	,,	85·4	15·0	0·8
B. 208	,,	79·0	12·1	1·8
P. O. J. 2878	,,	80·0	12·8	2·1
Co. 281	,,	84·5	13·6	0·9
Sugarcane × sorghum hybrids—				
Co. 351	,,	86·6	14·6	1·0
,, 352	,,	78·9	11·2	1·7
,, 353	,,	79·8	12·0	1·6
,, 354	,,	82·7	12·8	1·7
,, 355	,,	79·0	12·0	1·6
,, 356	,,	80·7	12·6	1·7
,, 357	,,	88·4	15·1	0·7
Anakapalle—September 1932 plantings.				
Other sugarcane varieties—				
M. A. 21	253	78·2	10·9	1·9
Co. 213	,,	64·5	6·9	3·2
,, 281	,,	72·5	9·4	1·4
Sugarcane × sorghum hybrids—				
Co. 351	,,	77·6	13·2	0·7
,, 352	,,	79·1	11·1	0·9
,, 353	,,	59·3	7·0	1·9
,, 354	,,	76·4	10·8	1·4
,, 355	,,	77·8	11·3	1·3
,, 356	,,	73·9	9·9	1·1
,, 357	,,	84·3	13·8	0·4
Palur—September 1932 plantings.				
Other sugarcane varieties—				
Co. 281	242	60·5	5·3	2·2
,, 213	,,	66·8	7·6	2·3
,, 290	,,	74·3	9·9	2·0
Sugarcane × sorghum hybrids—				
Co. 351	,,	70·0	8·6	2·5
,, 352	,,	68·5	7·7	3·1
,, 353	,,	68·1	7·0	3·3
,, 355	,,	70·4	9·6	2·9
,, 356	,,	67·3	9·0	3·3
,, 357	,,	81·7	12·5	1·9

TABLE XIII.

Showing periodical analyses of sorghum hybrids under different manurial treatments.

Field No. and treatment	Date of planting	Date of analysis	Age of the crop in days	Co. 352					Co. 353				
				Average wt. of a cane in lbs.	Brix	Sucrose	Purity	Glucose.	Average wt. of a cane in lbs.	Brix	Sucrose	Purity	Glucose
33 (100 lbs. nitrogen)	11th April 1932 .	21st December 1932.	253	2·07	14·18	11·05	77·9	1·18	2·75	16·48	13·69	83·1	0·94
	,, .	30th January 1933	293	2·92	19·42	17·39	89·5	0·29	2·34	18·99	16·27	85·7	0·69
	,, .	13th March 1933 .	335	2·46	19·12	16·36	85·6	0·45	2·98	18·19	15·36	84·5	0·77
56 (100 lbs. nitrogen)	20th April 1932 .	23rd December 1932	253	2·40	14·76	11·56	77·3	1·48	2·71	17·16	14·54	84·7	1·04
	,, .	2nd February 1933	293	2·56	19·89	17·39	87·4	0·33	2·86	19·79	17·39	87·8	0·59
	,, .	15th March 1933 .	335	2·62	21·59	19·76	91·5	0·52	3·54	20·67	18·50	89·5	0·50
24A (56 lbs. nitrogen)	14th April 1932 .	22nd December 1932	253	2·25	17·13	14·64	85·5	0·63	2·11	16·03	13·48	84·1	0·96
	,, .	31st January 1933	293	2·40	20·25	18·08	89·3	0·45	3·03	20·92	18·84	90·1	0·35
	,, .	14th March 1933 .	335	2·91	22·52	20·30	90·1	0·29	3·31	21·52	19·06	91·4	0·33

A PRELIMINARY NOTE ON THE MEMBRANEOUS BODY IN THE CYTOPLASM AS CHARACTERISTIC OF THE INDIGENOUS INDIAN CANES.

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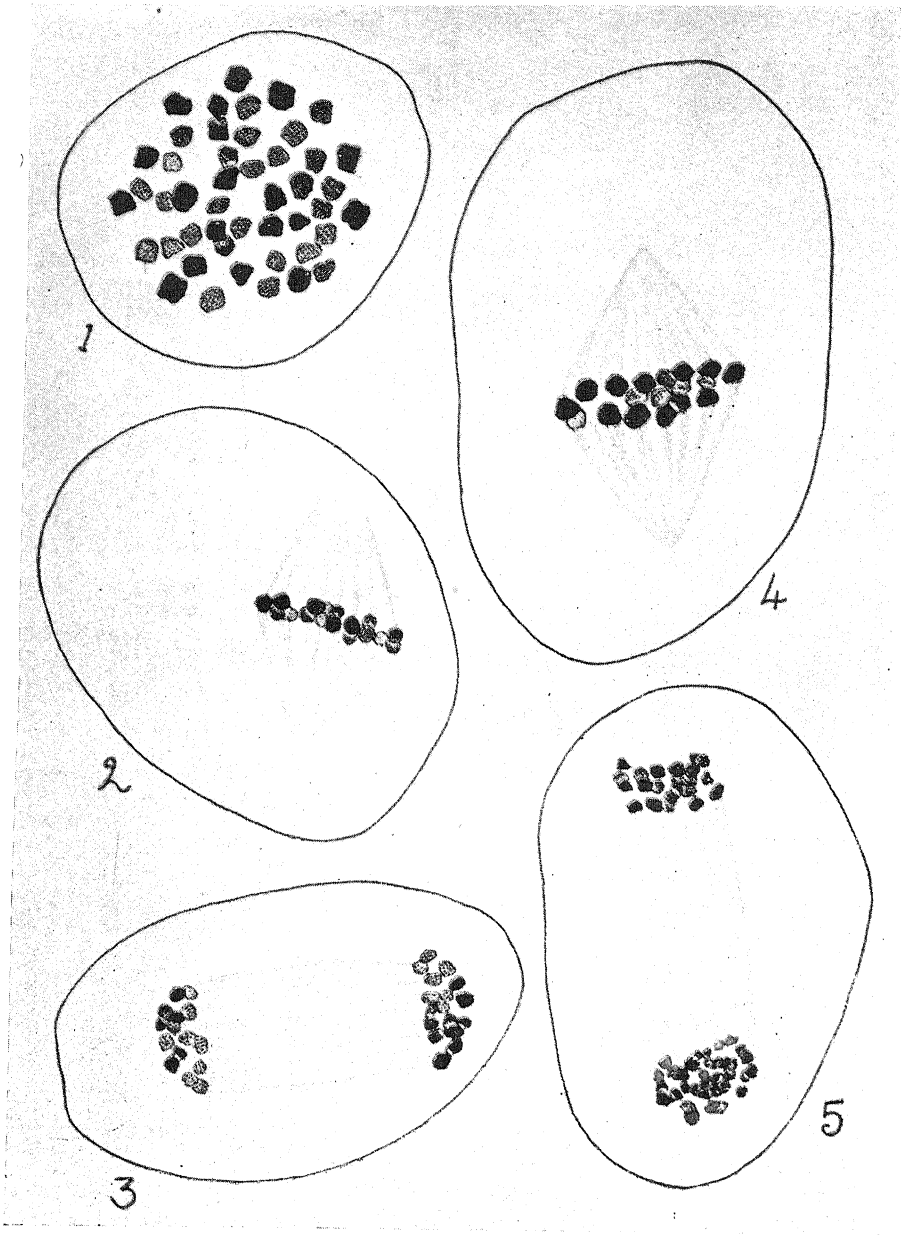
(With Plates XVI and XVII.)

The indigenous Indian canes were first exhaustively studied by Barber [1916] who, on their morphological characters, classified them into five separate groups, *viz.*, Mungo, Saretha, Sunnabile, Pansahi and Nargori. Jeswiet [1916] working in Java, classified the Pansahi group of Barber as *Saccharum sinense* (Roxb.), and placed the rest under a new species *Sacch. barberi* (Jesw.). Bremer [1932] has shown that the Indian canes differ in chromosome numbers not only from *Sacch. officinarum* and *Sacch. spontaneum*, but also among themselves, thus confirming Barber's classification of these canes into various groups. The diploid chromosome numbers in Mungo, Saretha, Sunnabile and Nargori, according to Bremer, are 80, 90-92, 116 and 124 respectively. Since these groups further possess distinct morphological characters which demarcate them from one another, Bremer doubted the desirability of placing all the four groups under one species *Saccharum barberi*.

According to Bremer, one interesting feature, characteristic of the whole Indian group of canes, including the Pansahi, is the presence of a long membraneous body in the cytoplasm (usually near one of the poles) during the metaphase and telophase of the reduction division. The presence of such a characteristic body would, no doubt, be a reliable criterion to separate the Indian forms from others.

The writers [Dutt and Subba Rao, 1933] had occasion to ascertain the chromosome numbers in certain sugarcane varieties that are being grown in the tropical parts of India and inci dentally noticed that the Coimbatore form of *Saccharum spontaneum* differed in chromosome number from the Java and the North Celebes forms mentioned by Bremer. The relationship of the size of nuclei of the microsporocytes to the haploid chromosome number in the various sugarcane varieties, including Saretha, was also studied. In the latter variety, while searching for the diakinesis stages, several other stages were met with and the absence of the characteristic body in all

PLATE XVI.



Figs. 1-3 are of Saretha, and 4 and 5 of Creole.
Note the absence of membraneous body in figs. 2-5 (×2,000).

the metaphase and the telophase stages was noted. It appeared worthwhile to pursue this point further, and material was, therefore, fixed in the following varieties during the current flowering season: Uba, Ketari, Merthi, Tekcha, Ohima (Pansahi group); Lalri, Kansar, Katha, Saretha (Saretha group); Hemja, Kharwi (Mungo group); Putli Khajee (Sunnabile group). The material of Saretha, collected last year, was fixed in Allen's modification of Bouin's fluid and the sections were stained in Haidenhain's iron alum hæmatoxylin. For purposes of comparison it was thought better to use the same fixative as that employed by Bremer and accordingly material was fixed this year in acetic alcohol.

The large number of sections that were cut last year in Saretha were again examined carefully for the presence of the body. The body was not noticed in any of the sections examined. Figs. 2 and 3 are camera lucida drawings of metaphase and telophase of Saretha, while Fig. 1 shows a metaphase plate of Saretha with 46 chromosomes. Before taking up the material fixed this year, through the usual grades of alcohol and xylol for embedding, a preliminary study was made of the same by staining in aceto-carmine. Fig. 6 shows a photomicrograph of one such variety, *i.e.*, Kansar, studied in this manner and the absence of the characteristic body in all the dividing pollen mother cells may be noted. Similarly a preliminary study by staining with aceto-carmine was made in all the other varieties fixed this year (representative of four of the groups of the indigenous Indian canes) and the body was again not traceable. To make sure about the correctness of the above observations, further detailed work is in progress.

An interesting corollary of Bremer's work regarding the presence of the membraneous body, was its application to the origin of the yellow Egyptian cane or Creole. Bremer noticed that the number of chromosomes in the somatic cells as also in the anaphase of the reduction division was 81, which was exactly the sum of the haploid number 41 of the Mungo group and 40 of *Sacch. officinarum*. The characteristic body was also noticed and that afforded Bremer a further indication of the Indian and hybrid origin of Creole. In the preparations of Creole made by the present writers last year, not a single pollen mother cell was met with that showed the characteristic body. Figs. 4 and 5 are camera lucida drawings of microsporocytes of Creole at metaphase and telophase.

From the above-mentioned preliminary observations, it will be seen that we have not so far noticed the characteristic body in either the indigenous Indian canes examined by us or in Creole, which is believed to contain the blood of a cane belonging to the Mungo group. If Bremer's observation on the presence of the membraneous body as characteristic of the whole Indian group of canes, could be confirmed, it would afford a valuable aid not only in identifying the Indian canes

cytologically, but also in tracing out their blood in the seedling cane varieties suspected or believed to have arisen from them.

We are indebted to Rao Bahadur T. S. Venkatraman for kindly placing the material of the Indian canes at our disposal. The Creole cane in the varietal plot at the Imperial Sugarcane Station, Coimbatore, was obtained through the courtesy of Mr. Noel Deerr in 1929.

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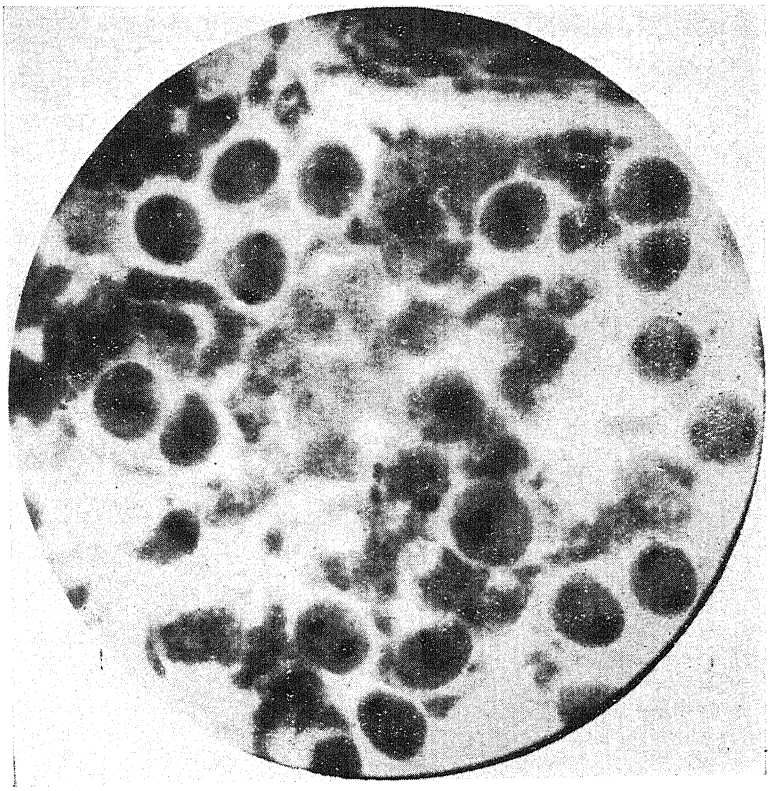
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(Translation of the Key to the Species of *Saccharum* from the above has appeared in *J. Agric. Res.* **39**, 1-30, 1929.)



Photomicrograph of dividing pollen mother cells of Kansar. Note the absence of membraneous body.



SELECTED ARTICLE

RESULTS OF STEM BORER EXPERIMENTS IN KRIAN DURING THE 1931-1932 PADI SEASON.

COMPILED BY

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FROM THE RECORDS OBTAINED BY

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(Reprinted from the *Malayan Agricultural Journal*, Vol. XXI, No. 8, August 1933.)

INTRODUCTION.

This report is based on the records left by Mr. H. T. Pagden, formerly Assistant Entomologist, to whom full credit must be given for all these records, since he was responsible for recording the data and for supervising the investigation of padi borers in the field.

The writer would like to place on record his appreciation of the work which Mr. Pagden has performed in endeavouring to obtain information for the control of padi borers during the past two years.

EXPERIMENTS.

Four experiments were laid down for the 1931-32 padi season at the following places in Krian :—Pagan Tiang (Swee Lee Estate), Titi Serong Experimental Station and 12th mile Bagan Serai.

EXPERIMENT I—YIELDS.

This experiment was for the purpose of comparing yields from non-colonised areas and areas colonised with the parasite, *Trichogramma minutum.*

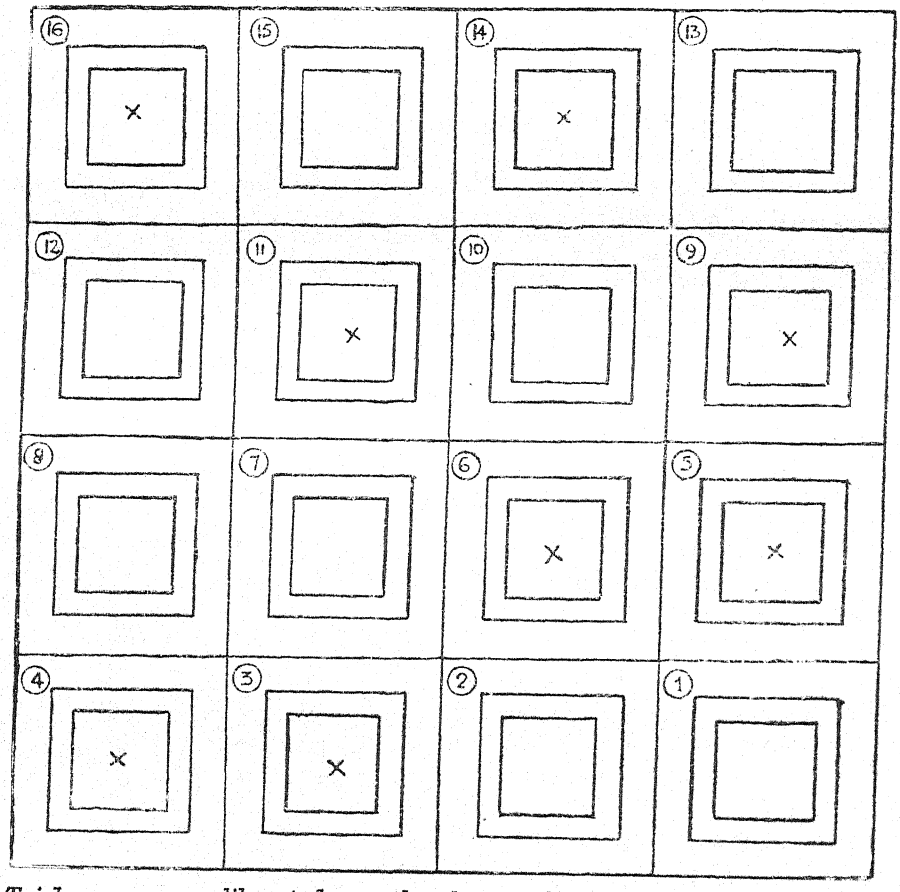
This area of 16 acres was situated on Swee Lee Estate, Mukim of Bagan Tiang. It was sub-divided into one-acre blocks. In the centre of each acre a 1/10 acre plot

was measured and over 8 of these 1/10 acre plots parasites were liberated at the rate of 20,000 per tenth acre. In the middle of each 1/10 acre plot a 1/40 acre plot was reserved for the purpose of calculating yields: stem counts were made from all 16 plots.

The type of padi used in this area was Seraup kechil 36. Seeds were sown on July 12th, 1931. The first transplanting was carried out on July 21st and the second on July 25th, 1931. The planting of the 16 acres commenced on September 28th and was completed on October 4th, 1931. The 1/40 acre plots were planted with 432 plants—a spacing of 18 inches.

No egg masses of stem-borer moths were collected from these plots.

DIAGRAM ILLUSTRATING THE ARRANGEMENT OF THE PLOTS IN EXPERIMENT I.



Trichogramma was liberated over the plots marked ×.

Liberation of Parasites :—The liberation of parasites commenced on December 3rd, 1931, in Plot 14 and ceased on December 18th, 1931, in Plot 11. Eight Plots, Nos. 3, 4, 5, 6, 9, 11, 14 and 16, were colonised and two circuits of liberations were made, 20,000 parasites being liberated in each 1/10 acre, equivalent to 200,000 per acre.

Stem Counts :—Stem counts were made from 1/10 acre plots. The work was commenced on January 25th. Twenty plants were taken from each 1/10 acre plot in one day, making altogether 320 plants from this area. Counting was commenced on January 26th and was completed on February 13th. The following figures show the result of the count from colonised and uncolonised areas :—

No. of tillers	No. of tillers attacked	Total No. of *Diatraea*, larvae and pupae found	Total No. of *Sesamia*, larvae and pupae found	Total No. of *Schoenobius*, larvae and pupae found
	Colonised Area			
1839 . . .	1358= 73·84 per cent.	395 larvae, 19 pupae	5 larvae	30 larvae
	Uncolonised Area			
1804 . . .	1393= 77·21 per cent.	816 larvae, 9 pupae	1 larva	60 larvae, 4 pupae

A very slight decrease in the percentage of attacked tillers in the Colonised area is revealed and more larvae of *Diatraea* and *Schoenobius* were formed in the Uncolonised than in the Colonised area.

Harvesting :—Harvesting of the 1/40 acre plots was commenced on February 22nd and was completed on February 25th.

The results are summarised thus :—

Plots receiving parasites :—493 plants (3456 planted) yielded about 3·5 gantangs of padi=0·11 paus per plant.

Plots not receiving parasites :—484 plants (3456 planted) yielded 3·6 gantangs of padi=0·12 paus per plant.

Out of 3456 plants planted only 493 in the Colonised area and 484 in the Uncolonised area remained. A considerable amount of rat damage occurred and this was followed by the growth of grasses and "menderong" which caused the padi to die. The plants harvested were alive but their appearance was very poor.

Experiment II—Natural and Induced Parasitism.

This area as situated adjacent to the area selected at Swee Lee Estate for Experiment I and the lay-out of this Experiment was similar to that described in Experiment No. 1. On eight 1/10 acre plots *Trichogramma* was liberated and coolies were employed to collect egg masses of stem borer moths from all the sixteen plots.

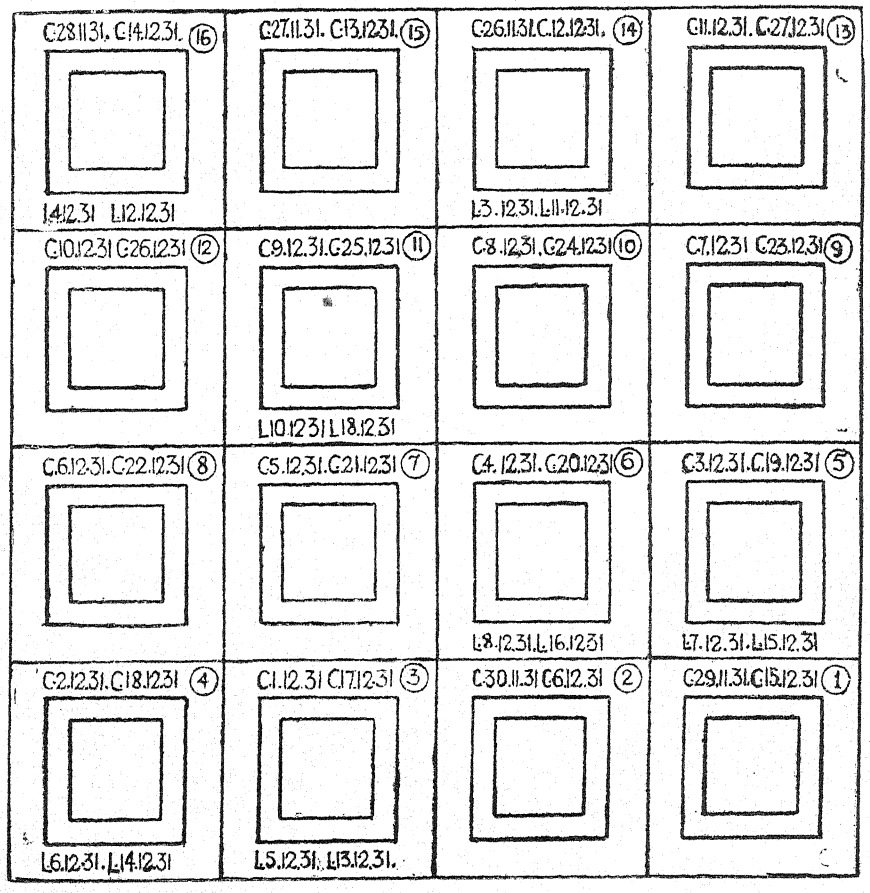
Seeds were sown on July 11th, 1931. The first transplanting was carried out on July 20th and the second on July 24th, 1931. Planting was commenced on September 22nd and was completed on October 10th, 1931.

Collection of Egg Masses :—Collection of egg masses was started on October 12th in Plot No. 1 and ceased on December 27th in Plot 13. The collection was discontinued when the padi was about to flower.

Egg masses of *Diatraea* were first collected on October 29th in Plot 2. *Schoenobius* first occurred on October 23rd in Plot 12. It is interesting to note that, although *Chilo* egg masses were collected in this area, they were not obtained in two other areas where egg collecting was carried out. *Chilo* egg masses were usually found on "menderong" (*Scirpus grossus*). *Diatraea* egg masses appeared to increase in December, the highest number collected in one day being 95 in Plot 15 on December 13th. *Schoenobius* remained more or less constant, the highest number being 24, collected from Plot 9 on December 23rd. The first *Chilo* egg

mass was collected in Plot 7 on October 18th. The largest number collected on one occasion was ten.

DIAGRAM ILLUSTRATING THE ARRANGEMENT OF THE PLOTS IN EXPERIMENT II.



C=Dates of collection of egg masses.
L=Dates of liberation of parasites.

Liberation of Parasites:—Liberation of parasites commenced on December 3rd, 1931, in Plot 14 and ceased on December 18th, in Plot 11. The liberation was so arranged to avoid liberations and collections in the same plot on the same day. The collection of ova continued until December 27th, so that egg masses from each plot were collected once after the liberation of parasites. Eight plots, Nos. 3, 4, 5, 6, 9, 11, 14 and 16, were colonised and two circuits of liberations

were made, 20,000 parasites being liberated altogether in each 1/10 acre plot equivalent to 200,000 per acre.

The results are tabulated herewith ;

(*I*) *Parasitism of Diatraea Egg Masses.*

Colonised Plots.			Uncolonised Plots.	
Plot No.	Percentage of Parasitism before liberation.	Percentage of Parasitism after liberation.	Percentage of Parasitism during first circuit.	Percentage of Parasitism during second circuit.
1	..	..	21·4	44
2	..	..	11·7	33·3
3	0	26·4	..	..
4	40	46·4	..	..
5	5·34	36·5	..	..
6	45	50	..	..
7	..	..	32·2	41
8	..	..	50	73
9	37	42·5	..	..
10	..	..	46·8	65·7
11	61·7	73	..	..
12	..	..	81·5	62·2
13	..	..	17·7	43·2
14	20	35·7	..	..
15	..	..	30	50·5
16	20	25	..	..

Colonised Area :—In the circuit immediately prior to the liberation of parasites, 192 egg masses, of which 78 were parasitised, *i.e.*, 40·6 per cent. parasitism, were collected from those plots intended for colonisation. After the liberation of *Trichogramma* 397 egg masses were collected from the colonised plots of which 158 were parasitised, a percentage of 39·8. This result indicates that the percentage parasitism was not increased by the introduction of parasites.

Uncolonised Area.—In the first circuit 304 egg masses were collected of which 142 were parasitised, giving a percentage parasitism of 46·7. In the second circuit 371 egg masses were collected of which 198 were parasitised, a percentage of 53·4.

The above figures show that an increase of about 7 per cent. in natural parasitism occurred during the interval between the first and second circuits in the uncolonised plots. This, however, was not recorded in the plots which received parasites.

(2) *Parasitism of Schoenobius Egg Masses.*

Colonised Plots.			Uncolonised Plots.	
Plot No.	Percentage of Parasitism by *Trichogramma* before liberation.	Percentage of Parasitism by *Trichogramma* after liberation.	Percentage of Parasitism by *Trichogramma* during first circuit.	Percentage of Parasitism by *Trichogramma* during the second circuit.
1	..	..	33·3	76·9
2	..	..	50	38·4
3	0	71·4	..	..
4	100	63·6	..	..
5	0	64·2	..	..
6	0	100	..	..
7	..	..	100	94·1
8	..	..	0	100
9	44·4	66·6	..	..
10	..	..	75·0	66·6
11	100	61·5	..	..
12	..	..	80	100
13	..	..	18·1	61
14	0	85·7	..	..
15	..	..	100	100
16	0	80	..	..

Colonised Plots.—Before liberation 22 egg masses were collected of which 9 were parasitised by *Trichogramma* giving a percentage parasitism of 40·9. After the liberation of parasites, 86 egg masses were collected of which 60 were parasitised by *Trichogramma*, a percentage of 69·8. This result indicates that the parasitism increased by 29. This, however, may be due to an increase in natural and not to induced parasitism (see below).

Uncolonised Plots.—During the first circuit 44 egg masses were collected of which 27 were parasitised by *Trichogramma*, giving a percentage parasitism of 61·4.

During the second circuit 101 egg masses were collected of which 74 were parasitised by *Trichogramma*, a percentage of 73·3. The natural parasitism would appear to have increased by about 12 per cent. indicating that the liberation of parasites was responsible for 17 per cent. of *Schoenobius* egg masses being parasitised.

(3) *Parasitism of Chilo Egg Masses.*

The number of *Chilo* egg masses collected from all 16 plots was 68. Only one and that from the Uncolonised area was parasitised by *Trichogramma.* This result indicates that *Trichogramma* is not an active parasite of *Chilo. Phanurus* emerged from 33 egg masses—a percentage of 48·5.

The following figures are the records of egg collections made during the period October 12th, 1931 to December 27th, 1931, from the Colonised and Uncolonised plots.

Diatraea.

Colonised Plots	Uncolonised Plots
Total No. of egg masses 664.	Total No. of egg masses 748.
Total No. of masses with *Trichogramma*, 256=38·6 per cent.	Total No. of masses with *Trichogramma*, 377=50 per cent.
Total No. of masses with 50 per cent. parasitism or over 193=29·1 per cent.	Total No. of masses with 50 per cent. parasitism or over 275=36·8 per cent.

Schoenobius.

Colonised Plots	Uncolonised Plots
Total No. of masses 120.	Total No. of masses 166.
Total No. of masses with parasites 82=68·3 per cent.	Total No. of masses with parasites 114=68·7 per cent.
Total No. with *Trichogramma* 69=57·5 per cent.	Total No. with *Trichogramma*, 98=59 per cent.
Total No. with *Phanurus* 8=6·7 per cent.	Total No. with *Phanurus* 5=3 per cent.
Total No. mixed with *Trichogramma* and *Phanurus* 5=4·2 per cent.	Total No. mixed with *Trichogramma* and *Phanurus* 11=6·6 per cent.

The liberation of *Trichogramma* over the Colonised Plots commenced on December 3rd, and continued until December 18th, so that these figures cannot be said to reveal the difference between the Colonised and the Uncolonised areas since the collection of egg masses was done only once after the liberation of *Trichogramma*.

Stem Counts.—Stem counts were made from all 1/10 acre plots. The work was commenced on January 20th. Twenty plants were taken from each 1/10 acre plot on one day, making altogether 320 plants from this area. Counting was commenced on January 26th and was completed on February 13th. The following figures show the results of the counts of stems from the Colonised and Uncolonised areas :—

Colonised Area		Uncolonised Area	
No. of tillers	2,368	No. of tillers	2,508
No. of tillers bored 1,687=71·2 per cent.		No. of tillers bored 1,693=67·50 per cent.	
No. of *Diatraea* larvae	586	No. of *Diatraea* larvae	663
No. of *Diatraea* pupae	25	No. of *Diatraea* pupae	9
No. of *Sesamia* larvae	9	No. of *Sesamia* larvae	7
No. of *Sesamia* pupae	*Nil*	No. of *Sesamia* pupae	*Nil*
No. of *Schoenobius* larvae	29	No. of *Schoenobius* larvae	36
No. of *Schoenobius* pupae	1	No. of *Schoenobius* pupae	2

This result shows a greater percentage of bored tillers in the Colonised than in the Uncolonised area.

Harvesting.—Harvesting of the 1/40 acre plots was started on February 14th, 1932, in Plots Nos. 13 and 14 and was completed on February 20th, 1932.

The yields from plants in the Colonised and Uncolonised plots were as follows :—

Colonised Area					Uncolonised Area				
Plot No.	Yield				Plot No.	Yield			
	Gantang	Chupak	Paus	No. of plants		Gantang	Chupak	Paus	No. of plants
3	5	1	1	374	1	6	2	1	408
4	3	3	0	399	2	6	0	3	413
5	2	1	1	365	7	4	1	2	360
6	1	3	1	375	8	1	3	0	381
9	2	2	2	370	10	1	3	3	363
11	0	3	0	182	12	0	3	0	313
14	2	0	2	360	13	3	2	3	362
16	3	2	0	369	15	3	3	0	384
	22	0	3	2,794		29	0	0	2,984

Plots receiving parasites.—2,794 plants (3,456 planted) yielded about 22 gantangs of padi=0·13 paus per plant.

Plots not receiving parasites.—2,984 plants (3,456 planted) yielded 29 gantangs of padi=0·16 paus per plant. This result shows a lower yield from those plots over which parasites were liberated.

It will be seen that out of 3,456 plants planted only 2,794 and 2,984 remained in the colonised and uncolonised plots respectively. A considerable amount of rat damage occurred which was followed by the appearance of grasses and 'menderong' causing the padi to die.

EXPERIMENT III—SEASONAL OCCURRENCE OF BORERS.

The purpose of this experiment was to ascertain whether stem borers were seasonal. Last season's work indicated that irrespective of the condition of padi, stem borers may be seasonal.

Schoenobius became numerous in late December, 1930, *Diatraea* in January, 1931, when collection ceased.

Two 1/6 acre plots were reserved, one at Swee Lee Estate and the other 13 miles away in a south easterly direction at 12th mile, Bagan Serai. The type of padi used was Seraup Kechil 36 at Swee Lee Estate and Seraup Besar at Bagan Serai. It was intended to plant the same variety, but, since all the padi had been planted at Bagan Serai, there was no alternative but to select 1/6 acre which had been planted with Seraup Besar.

Five coolies were employed at each area for collecting egg masses until the padi plants died.

The collection of egg masses was commenced at both places on October 12th, 1931, and ceased on March 31st, 1932.

The figures below show the total number of egg-masses collected in each month.

Swee Lee Estate			Bagan Serai		
Period	*Diatraea*	*Schoenobius*	Period	*Diatraea*	*Schoenobius*
October 31 . .	45	3	October 31 . .	42	103
November 31 . .	153	12	November 31 .	507	563
December 31 . .	1,389	71	December 31 .	570	279
January 32 . .	901	16	January 32 . .	285	101
February 32 . .	105	0	February 32 . .	531	17
March 32 . .	432	27	March 32 . .	437	7
Total .	3,025	129	Total .	2,372	1,070

The above figures show that the largest number of *Diatraea* egg masses collected from both areas was in December, 1931, with 1389 at Swee Lee Estate, and 570 at Bagan Serai. The minimum number collected from both areas was in October with 45 masses at Swee Lee and 42 at Bagan Serai. One egg mass of *Diatraea* was collected at Bagan Serai on the day the collection commenced (October 12th) but at Swee Lee none was collected until October 17th.

Egg masses of Diatraea at Swee Lee Estate.—During October 45 egg masses of *Diatraea* were collected at Swee Lee, 10 being the maximum in one day. During most of November the numbers remained constant but at the end an increase occurred. The number of egg masses remained high throughout December and commenced to decrease at the beginning of January but there was a large rise in the last week of January. The number of egg masses was low throughout February and this was maintained until about the last week of March, when an increase occurred.

Egg masses of Diatraea at Bagan Serai.—The number of egg masses collected was low throughout October. A marked increase occurred about the third week of November. During December the number fluctuated but decreased considerably towards the end of the month and during January. An increase commenced at the beginning of February, remaining fairly high throughout the month. Towards the end of March the numbers again increased.

There would appear to be evidence to suggest that *Diatraea* moths are more numerous in some months than in others. From both these areas the largest number of eggs was collected in December. Results in 1930-31 however, showed that the increase occurred in February.

Schoenobius at Swee Lee.—The egg masses for *Schoenobius* in this area were lower than at Bagan Serai throughout the season. The largest number (71) was collected in December and 10 was the largest number collected on one day (December 13th). An increase occurred in March.

Schoenobius at Bagan Serai.—The largest number (563) was collected in November. The largest number collected in one day was 61 on December 12th. From this month the collections decreased, only 7 masses were collected in March. In 1930-31 season, most *Schoenobius* egg masses were collected about the end of January and the beginning of February but this season's collections show that at Swee Lee and Bagan Serai, November for the former and December for the latter were the peak months and not January and February. Considerably more work is undoubtedly required to ascertain the causes responsible for seasonal fluctuations.

The natural parasitism of the egg masses collected at Swee Lee Estate and Bagan Serai gave the following result.

1. Diatraea Egg Masses.

Period	Total No. of masses	Total No. of masses with *Trichogramma*	Per cent.	Total No. of masses with 50 per cent. parasitism or over	Per cent.
October	45	*Nil*	—	*Nil*	—
November	153	3	2·0	3	2·0
December	1,389	184	13·2	64	4·6
January	901	418	46·4	225	25·0
February	105	48	45·7	32	30·5
March	432	190	44·0	113	26·2
Total .	3,025	843		437	
October	42	2	4·8	1	2·4
November	507	106	20·9	24	2·7
December . . .	570	231	40·5	100	17·5
January . . .	285	83	29·1	27	9·5
February . . .	531	48	9·0	11	2·1
March	437	180	41·2	113	25·9
Total .	2,372	650		276	

At Swee Lee the total number of egg masses collected was 3,025 out of which 843 were parasitised by *Trichogramma*, a percentage parasitism of 27·9. Out of the 843 masses, 437 (=14·4 per cent.) showed 50 per cent. parasitism or over.

At Bagan Serai the total number of *Diatraea* egg masses collected was 2,372, of which 650 (=27·4 per cent.) were parasitised by *Trichogramma*. Out

of the 650 masses, 276 (=11·6 per cent.) showed a 50 per cent. parasitism or over.

The largest number of parasitised egg masses at Swee Lee occurred in January; out of 901 egg masses, 418 were parasitised and 225 of these showed 50 per cent. parasitism or over. The parasitism during January and March remained about 40 per cent. At Bagan Serai the parasitism was lower than at Swee Lee and parasitism only in December and March reached 40 per cent. During March 437 egg masses were collected, 180 contained parasites of which 113 showed a 50 per cent. parasitism or over.

Out of the total number of *Diatraea* egg masses collected at Swee Lee and Bagan Serai only one egg mass collected at Bagan Serai was parasitised by *Phanurus beneficiens*, so that this parasite is not an active enemy of *Diatraea*.

2. Schoenobius Egg Masses.

Period	Swee Lee Estate						
	Total No. of masses	Total No. of masses with parasites	Total No. of masses with Trichogramma	Total No. of masses with Phanurus	Total No. of masses mixed with Tric. and Phanurus.	Total No. of masses with Tetrastichus	Total No. of masses mixed with Tetrastichus and Trichogramma.
October	3	—	—	—	—	—	—
November	12	1	—	1	—	—	—
December	71	14	13	1	—	—	—
January	16	8	6	—	2	—	—
February	0	—	—	—	—	—	—
March	27	18	10	2	1	4	1
	129	41	29	4	3	4	1

2. Schoenobius Egg Masses—contd.

Period	Bagan Serai.							
	Total No. of masses	Total No. of masses with parasites	Total No. of masses with Trichogramma	Total No. of masses with Phanurus	Total No. of masses mixed with Tric. and Phanurus	Total No. of masses with Tetrastichus	Total No. of masses mixed with Tet. and Tric.	Total No. of masses mixed with Tetra. and Phanurus
October . . .	103	7	—	7	—	—	—	—
November . . .	563	140	38	89	13	—	—	—
December . . .	279	135	46	98	41	—	—	—
January . . .	101	72	7	44	13	3	3	2
February . . .	17	5	1	2	1	1	—	—
March . . .	7	4	1	1	1	1	—	—
	1,070	143	93	241	69	5	3	2

At Swee Lee the total number of *Schoenobius* egg masses collected was 129 out of which 41 were parasitised.

88 not parasitised . . . . . 68·2 per cent.
29 masses by *Trichogramma* . . . . 22·5 ,, ,,
4 masses by *Phanurus* . . . . . 3·1 ,, ,,
3 masses by *Trichogramma* and *Phanurus* . . 2·3 ,, ,,
4 masses by *Tetrastichus* . . . . . 3·1 ,, ,,
1 mass by *Tetrastichus* and *Trichogramma* . . 1·0 ,, ,,

At Bagan Serai the total number of *Schoenobius* egg masses collected was 1,070, out of which 413 were parasitised as follows :—

657 masses unparasitised . . . . . 61·4 per cent.
93 masses by *Trichogramma* . . . . . 8·7 ,, ,,
241 masses by *Phanurus* . . . . . 22·5 ,, ,,
69 masses by *Trichogramma* and *Phanurus* . . 6·4 ,, ,,
5 masses by *Tetrastichus* . . . . . 0·5 ,, ,,
3 masses by *Tetrastichus* and *Trichogramma* . . 0·3 ,, ,,
2 masses by *Tetrastichus* and *Phanurus* . . 0·2 ,, ,,

The majority of the egg masses at Swee Lee were parasitised by *Trichogramma* and the majority at Bagan Serai by *Phanurus*. The highest percentage parasitism occurred both at Swee Lee and Bagan Serai in January, 50 at the former and 71 at the latter area.

Towards the end of February most of the padi plants in both the 1/6 acre at Swee Lee and Bagan Serai were dead and the collection of egg masses was confined to some of the remaining fresh plants and volunteer padi. During March, all the padi plants in the two areas were dead and the collection of egg masses was limited to volunteer padi.

Experiment IV—Light Traps.

Three light traps were set up in the Government Padi Experimental Station at Titi Serong. Ordinary kerosene-burning hurricane lanterns were used as the source of light. The moths were trapped in trays 2 feet square and 3 inches deep, containing water covered with a film of kerosene. Each trap was protected from rain by a roof constructed from nipah leaves.

Two lamps were lit for a part of the night, the third burning all night. The hours during which the lamps were kept alight were as follows :—

No. 1—10-00 p. m. to 6 a. m.

No. 2—6-30 p. m. to 10 p. m.

No. 3—6-30 p. m. to 10 p. m.

The records for this season were taken from June 15th, 1931, to March 31st, 1932. The above arrangement of the light was carried out from June 15th to 31st July, 1931, when only two of the lamps, which were kept alight all night from 6-30 p. m. to 6 a. m., were used.

The results obtained with each of the three species of borer are given separately below :—

Diatraea auricilia :—From the night of the 15th-16th June to the night of 29th-30th June very few moths were caught. The maximum number of males and females respectively was 4 and 5. During July no moths were obtained. In August, when only two lamps were used a few moths were obtained during the first and second weeks of the month. Moths were absent again from the night of 13th-14th August till the night of 18th-19th August when a male was caught and another on the night of 22nd-23rd August. During September moths appeared at the end of the first week and were absent again about the end of the month. During October no moths were caught until the third week of the month. In November very few were captured but a slight increase of the females of the species occurred at the end of the month. During December

an increase in males and females occurred but the number of males decreased in the latter part of the month. In January *Diatraea* increased, the maximum number of males and females caught in one night was 89 and 102 respectively.

At the beginning of February about the same number of males and females was caught each night, but towards the end of the month a marked increase in the number of females occurred. The largest number of *Diatraea* caught in one night was 571. In March the number of males dropped and of females increased considerably. The maximum number of females caught in one night was 588, the highest number recorded during the season.

The figures below show the catch for each month :—

Period	Males	Females
June 15th to 30th inclusive, 1931	15	16
July	*Nil*	*Nil*
September	31	10
October	15	1
November	30	36
December	239	291
January—1932	601	632
February	629	1,883
March	415	3,770

The total number of moths caught from the night of June 15th-16th to and including the night of March 30th-31st, 1932, was males 1,975 and females 6,639.

Schoenobius incertellus :—The first considerable increase in the number of moths occurred in December. The total number for the month was 420 males and 105 females. During June, July, August, September, October and November very few moths were captured.

The largest number recorded during the season was 422 males caught in February and 254 females caught in March. The major difference between *Diatraea* and *Schoenobius* at light is that the females of the former and males of the latter predominated.

The total number of moths caught in each month during the season was as follows :—

Schoenobius incertellus.

Period	Males	Females
June 15th to 30th inclusive	19	6
July	*Nil*	*Nil*
August	3	15
September	50	12
October	15	1
November	31	9
December	420	105
January	377	111
February	422	124
March	31	254

The total number of *Schoenobius* moths caught during the season was 1,368 males and 637 females.

Sesamia inferens :—*Sesamia* first appeared in the traps in December, 1931. From December, 1931 to March, 1932, the total number caught was 92 males and 4 females.

The table below shows the number caught in each month during the season.

Sesamia inferens.

Period	Males	Females
June 15th to 30th inclusive	*Nil*	*Nil*
July	*Nil*	*Nil*
August	*Nil*	*Nil*
September	*Nil*	*Nil*
October	*Nil*	*Nil*
November	*Nil*	*Nil*
December	5	*Nil*
January	5	*Nil*
February	42	1
March	40	3

These results differ considerably from those of 1930-31. In the case of *Diatraea* very few were attracted to the light before February, whereas in 1931-32 a marked increase was recorded in December and continued till the termination of this experiment at the end of March. Few *Schoenobius* moths before January 1931 were attracted but this season's result shows a large increase in December. A marked decrease was recorded in March in this season's work, but in 1930-31 the decrease was not recorded till April. It is suggested that these variations may be due to padi being planted earlier in the 1931-32 than in the 1930-31 season but no definite statement can be made at this stage especially since Mr. Pagden is of the opinion that *Diatraea* is to some extent seasonal in the Krian District as evidenced by the usually good crop obtained by early planting even when the area is adjacent to one planted later and subsequently subject to heavy attack.

SUMMARY.

EXPERIMENT I.

STEM COUNTS.

About the same number of tillers was attacked in the Colonised and Uncolonised areas but the stems in the Uncolonised area contained more larvae and pupae than in the Colonised area.

Trichogramma cannot be stated to have prevented the boring of stems.

EXPERIMENT II.

1. NATURAL AND INDUCED PARASITISM.

Diatraea.

In the plots receiving parasites, no increase of parasitism after the liberation of parasites was recorded, but in the Uncolonised plots an increase of about 7 per cent. in natural parasitism occurred.

Schoenobius.

In the Colonised plots the percentage parasitism increased by 29 per cent.

In the Uncolonised area, natural parasitism increased by 12 per cent., indicating a possible increase due to *Trichogramma* of 17 per cent. : such a deduction, however, is not supported by the result obtained in the *Diatraea* collection.

Chilo.

Trichogramma is not an active parasite on the eggs of this moth. This parasite only emerged from one egg mass out of the 68 collected.

2. Stem Counts.

A larger percentage of stems was bored in the Colonised than in the Uncolonised areas.

3. Harvesting.

A larger yield was obtained from the Uncolonised than from the Colonised areas.

Experiment III.—Seasonal occurrence of borers.

1. Diatraea.

Swee Lee—Diatraea increased at the end of November and continued during December. An increase was also recorded in March.

Bagan Serai—Diatraea increased towards the end of November, decreased considerably towards the end of December and increased again in February.

The increase during 1930-31 padi season occurred in February.

Diatraea is more numerous in certain months and there would appear grounds for supposing that it may be seasonal as this result indicates that more egg masses were collected at Bagan Serai about a month earlier than at Swee Lee.

2. Schoenobius.

Schoenobius was more numerous at Bagan Serai than at Swee Lee and would appear to be more prevalent at Bagan Serai a month earlier than at Swee Lee.

3. Trichogramma.

Trichogramma from the *Diatraea* egg masses was obtained in October at Bagan Serai and in November at Swee Lee and from *Schoenobius* in November at Bagan Serai and December at Swee Lee.

Experiment IV.—Light traps.

Moths were attracted to light traps a month earlier than in 1930-31.

It is impossible to say whether these moths are seasonal but increases seem to occur as the supply of food material increases.

Discussion.

The 1931-32 experiments have revealed that the study of these borers is attended with endless difficulties and that considerably more work will be required before efficient measures for their control can be recommended. The results of the investigations made during the past two years indicate that Trichogramma cannot be considered as an effective parasite in the control of padi stem borers even when liberated in large numbers. The liberations made during the 1930-31 padi season, recorded in *Malayan Agricultural Journal*, March 1932, showed a marked increase in parasitised ova but in the case cited the total rate of liberation was 1,300,000 parasites per acre, parasites being liberated daily from the beginning of the padi season until ripening of the grain, an impossible procedure where large areas may have to be colonised. Furthermore, it should be noted that only *Diatraea* was attacked to any extent, *Schoenobius* being nearly immune owing to the hairy covering of the egg masses.

In the experiments recorded above an attempt was made to put the liberation of parasites on a more economic basis by liberating in smaller numbers for a short period at the first sign of an increase in borer activity, but even here, although 200,000 parasites per acre were used, the results demonstrate that in these numbers the parasite is ineffective.

NOTE

IMPORTATION OF PLANTS INTO ENGLAND AND WALES.

1. The Ministry of Agriculture and Fisheries, England, has amended the regulations affecting the importation of plants into England and Wales, contained in the Destructive Insects and Pests Order of 1922. The revised order called the Importation of Plants Order of 1933, which came into force on the 15th July 1933, is published below for general information. An Order in identical terms applicable to Scotland has been made by the Department of Agriculture for Scotland.

2. The principal alterations involved by the new Order are :—

(1) The requirement of a certificate of health is no longer limited to plants with a persistent woody stem, but applies to all living plants and parts thereof (except seeds) for planting.

(2) Consignments which arrive unaccompanied by the prescribed certificate will not be allowed as heretofore to proceed to their destination, but will be detained at the port of the entry until their release has been authorised.

(3) The schedule of pests embodied in the Destructive Insects and Pests Order of 1922 has been dropped : plants must be certified as having been "found to be healthy, no evidence of the presence of any insect, fungus or pest destructive to agricultural or horticultural crops having been found in them".

(4) The additional certificate required for potatoes must state that no case of Wart Disease has occurred within two kilometres of the place where the potatoes were grown, instead of 500 yards as heretofore.

(5) Health certificates are no longer required for onion and leek seeds or for gooseberries (fruit).

3. The arrangements now in force (published in paragraph 2 of the Government of India Education, Health and Lands Department Notification No. 360, dated the 29th February, 1924) for the inspection and certification of plant consignments intended for export from this country to the United Kingdom and the Irish Free State will continue. The Secretary, Agricultural and Horticultural

Society of India, Alipore, Calcutta, has also since been authorised to issue the necessary certificates in respect of plants exported through the port of Calcutta. The Society will levy a fee at 5 per cent. on the invoice value of the consignment subject to a minimum charge of Rs. 5. Exporters from the provinces adjacent to Bengal may also obtain the requisite certificate from the Secretary of this Society on payment of the prescribed fee.

STATUTORY RULES AND ORDERS,
1933, No. 558.

DESTRUCTIVE INSECT AND PEST, ENGLAND.

THE IMPORTATION OF PLANTS ORDER OF 1933. DATED JUNE 7, 1933.

(D. I. P. 567.)

The Minister of Agriculture and Fisheries by virtue and in exercise of the powers vested in him under the Destructive Insects and Pests Acts, 1877 to 1927, (*a*) and of every other power enabling him in this behalf, orders as follows :—

Definitions.

1. In this Order, unless the context otherwise requires :—

"Importer" includes any person who, whether an owner, consignor or consignee, agent or broker, is in possession of, or in anywise entitled to the custody or control of, the article.

"Inspector" means an Inspector or other authorised officer of the Ministry of Agriculture and Fisheries.

"Minister" means the Minister of Agriculture and Fisheries.

"Plant" includes tree and shrub, and the fruit, seeds, tubers, bulbs, corms, rhizomes, roots, layers, cuttings and other parts of a plant.

"Raw Vegetables" includes raw onions, raw tomatoes, raw aubergines and raw salads.

"Unhealthy" means affected with any insect, fungus or other pest destructive to agricultural or horticultural crops.

Application of Order.

2. Nothing in this Order shall be deemed to prohibit or restrict the landing or transhipment in England or Wales of any produce grown in Scotland, Northern Ireland, the Irish Free State, the Isle of Man or the Channel Islands.

(*a*) 40-I. V. c. 68, 7 E. 7. c. 4 and 17-8 G. 5. c. 32.

Prohibition against landing and restriction on transhipment in England or Wales of certain Potatoes.

3. (1) The landing in England or Wales of any potatoes grown in the undermentioned countries is prohibited :—

the United States of America, the Dominion of Canada and European France.

(2) The transhipment in England or Wales of any of the potatoes specified in sub-section (1) of this Article is prohibited except under and in accordance with the conditions of a licence issued by the Minister or by an Inspector.

(3) In this Article "potatoes", includes potato haulms, leaves and stalks.

Restrictions on landing in England or Wales of certain Plants and Potatoes.

4. (1) The landing in England or Wales of any of the plants mentioned in the First Schedule to this Order and of potatoes is hereby prohibited, unless each consignment is accompanied by two copies of a certificate of a duly authorised Official of the Phytopathological Service of the country in which the plants or potatoes were grown in the form prescribed in the Second Schedule to this Order. The inspection referred to in the certificate shall be carried out not more than fourteen days prior to the date of shipment. The original of the certificate shall be forwarded by post by the exporter to the Horticulture Branch of the Ministry of Agriculture and Fisheries, 10, Whitehall Place, London, S. W. 1, before the plants or potatoes are despatched. Except in the case of consignments imported through the post, a copy of the prescribed certificate shall be delivered to an Officer of Customs and Excise at the same time as and together with the entry relating to the consignment. In the case of consignments imported through the post, a copy of the prescribed certificate shall be affixed to each package.

(2) In the case of any of the plants mentioned in the First Schedule and grown in European France, the certificate referred to in this Article shall include a statement by an Officer of the French Services d'Inspection Phytopathologique in the form set out in the Third Schedule to this Order.

(3) Nothing contained in this Article shall be deemed to permit the landing or transhipment in England or Wales of any potatoes of which the landing or transhipment is prohibited or restricted under Article 3 hereof.

Restrictions on the landing in England or Wales of Raw Vegetables.

5. (1) The landing in England or Wales between the fifteenth day of March and the fourteenth day of October in any year of any raw vegetables grown in European France is hereby prohibited unless each consignment is accompanied by a certificate issued by an Officer of the French Services d'Inspection Phytopathologique in the form set out in the Third Schedule to this Order.

(2) The landing in England or Wales between the fifteenth day of March and the fourteenth day of October in any year of any raw vegetables not grown in European France is hereby prohibited unless each consignment is accompanied by a certificate of origin visé by a competent authority in the country of origin, stating the country and place where the produce was grown. The certificates prescribed in this Article shall be delivered to an Officer of Customs and Excise at the same time as and together with the entry relating to the consignment.

(3) Nothing contained in this Article shall be deemed to permit the landing or transhipment in England or Wales of any potatoes of which the landing or transhipment is prohibited or restricted under Article 3 hereof.

Restrictions on the landing in England or Wales of certain raw apples.

6. (1) The landing in England or Wales between the fifteenth day of March and the fourteenth day of October in any year of any cider apples grown in any European country other than France is hereby prohibited unless each consignment is accompanied by a certificate of origin visé by a competent authority in the country of origin, stating the country and place where the apples were grown.

(2) The landing in England or Wales between the fifteenth day of March and the fourteenth day of October in any year of any cider apples grown in European France is hereby prohibited unless each consignment is accompanied by a certificate issued by an Officer of the French Services d'Inspection Phytopathologique in the form set out in the Third Schedule to this Order.

(3) The landing in England or Wales between the seventh day of July and the fifteenth day of November in any year of any raw apples grown in the United States of America is hereby prohibited unless each consignment is accompanied by a certificate signed by a duly authorised Inspector of the Federal Department of Agriculture in the form set out in the fourth Schedule to this Order.

(4) The certificates prescribed in this Article shall be delivered to an Officer of Customs and Excise at the same time as and together with the entry relating to the consignment.

Procedure where Plants, Potatoes, Raw Apples or Raw Vegetables are landed in contravention of this Order.

7. (1) If plants, potatoes, raw apples or raw vegetables are landed in England or Wales in contravention of this Order, they shall forthwith be destroyed or re-exported at the expense of the importer, unless they are otherwise disposed of in accordance with the terms of a licence issued by the Minister or by an Inspector.

(2) Such licence may provide for the examination of any such plants, potatoes, raw apples, or raw vegetables, and after examination thereof, the Inspector shall give a certificate stating whether the plants, potatoes, raw apples or raw vegetables or any of them are unhealthy or not. Such plants, potatoes, raw apples or raw vegetables as are so certified to be unhealthy shall be dealt with in accordance with Article 9 hereof as the Minister or the Inspector may direct.

(3) The importer shall pay for the said certificate such fee as the Minister may determine not exceeding five pounds.

Powers of Inspector in respect of consignments of plants, potatoes, raw apples or raw vegetables.

8. An Inspector, upon production if so required of his appointment or authority, may enter any premises and examine and take samples of any consignment or part of a consignment of plants, potatoes, raw apples or raw vegetables landed or suspected of having been landed in England or Wales, notwithstanding that such consignment was accompanied by such certificates as are required by this Order. For the purpose of any examination under this Article, an Inspector may open, or require the importer to open, any package in the consignment.

Procedure in the case of unhealthy consignments of plants, potatoes, raw apples or raw vegetables.

9. (1) If it appears to an Inspector at any time that any plants, potatoes, raw apples, or raw vegetables in any consignment or part of a consignment landed in England or Wales, are unhealthy, he may serve upon the importer a notice stating that such plants, potatoes, raw apples or raw vegetables have been found by him to be unhealthy, and requiring the importer, within

such time as may be specified in the notice, to carry out such measures of disinfection or treatment as may be specified in the notice, or to destroy or re-export the whole or any part of the consignment.

(2) Any measure of disinfection or treatment required by a notice under this Article shall be carried out by the importer upon whom the notice is served, under the supervision of an Inspector at a suitable place designated by the Inspector, and no part of the consignment shall be moved from the place designated without the written authority of the Minister or the Inspector.

(3) Charges for or in connection with the removal, storage, disinfection, treatment, destruction or re-exportation, required by any such notice, shall be paid by the importer on whom the notice is served.

(4) In this Article "consignment" includes packing materials, cases and containers.

Licences.

10. Notwithstanding any provisions of this Order, any plants, potatoes, raw apples, or raw vegetables may be landed in England or Wales under and in accordance with the conditions of a licence issued by the Minister or by an Inspector.

Service of Notices, etc.

11. For the purpose of this Order, a notice shall be deemed to be served on any person if it is delivered to him personally or left for him at his last known place of abode or business or sent through the post in a letter addressed to him there ; and a notice purporting to be signed by an Inspector shall be *primâ facie* evidence that it was signed by him.

Information to be given as to plants, potatoes, raw apples or raw vegetables landed in England or Wales.

12. Every person who has or has had in his possession or under his charge any plants, potatoes, raw apples or raw vegetables landed in England or Wales, and every person who as auctioneer, salesman or otherwise, has sold or offered for sale any such plants, potatoes, raw apples or raw vegetables shall, if so required in writing by the Minister or an Inspector, give to the Minister or Inspector all such information as he possesses as to the persons who have or have had in their possession or under their charge such plants, potatoes, raw apples, or raw vegetables, provided that any information given under this Article shall not be available as evidence against the person giving the same in any prosecution under this Order, except in respect of an alleged failure to comply with this Article.

Offences.

13. Every person shall be liable on conviction to a penalty not exceeding ten pounds, or in respect of a second or subsequent offence to a penalty not exceeding fifty pounds who wilfully obstructs or impedes an Inspector in the exercise of his powers under this Order or who does any act in contravention of this Order or of the terms or conditions of any notice or licence served or issued thereunder, or fails to do any act which he is required to do by this Order or any such notice or licence.

This Article does not apply to the landing or attempted landing of anything in contravention of this Order, the penalty for which is provided by section 1 of the Destructive Insects Act, 1877, as amended by the Destructive Insects and Pests Acts, 1907 and 1927.

Revocation of Orders.

14. The Orders specified in the Fifth Schedule to this Order are hereby revoked.

Provided that the revocation shall not—

(*i*) affect the previous operation of such Orders or anything duly done or suffered under such Orders ; or

(*ii*) affect any right, privilege, obligation or liability acquired, accrued or incurred under such Orders ; or

(*iii*) affect any penalty incurred in respect of any offence committed against such Orders ; or

(*iv*) affect any investigation, legal proceeding or remedy in respect of any such right, privilege, obligation, liability or penalty as aforesaid ; and any such investigation, legal proceeding or remedy may be instituted, continued, or enforced, and any such penalty may be imposed as if this Order hadnot been made.

Commencement.

15. This Order shall come into operation on the fifteenth day of July, nineteen hundred and thirty-three.

Short Title.

16. This Order may be cited as the Importation of Plants Order of 1933.

In witness whereof the Official Seal of the Minister of Agriculture and Fisheries is hereunto affixed this seventh day of June, nineteen hundred and thirty-three.

(L.S.)

H. E. DALE,

Principal Assistant Secretary.

FIRST SCHEDULE.

All living plants and parts thereof (except seeds) for planting.

SECOND SCHEDULE.

This is to certify that { the living plants / a representative sample of the living plants

(*Strike out the words not applicable.*)

included in the consignment, of which particulars are given below, were/was thoroughly examined on the..... (date) by..... (name) a duly authorised Official of the.....and found to be healthy, no evidence of the presence of any insect, fungus or pest destructive to agricultural or horticultural crops having been found in them.

This additional certificate must be given for all potatoes :—

It is further certified that no case of the disease known as "wart disease" or "black scab" of potatoes (*Synchytrium endobioticum*) has occurred at any time on the farm or holding where the potatoes included in the consignment were grown, nor within two kilometres thereof.

(Signed).....

(Official status).....

No. and description of packages.....
Distinguishing marks.....
Description of living plants or parts thereof.....
.....
Stated to be grown at.....
Exported by.....
Name and address of consignee.....
.....
Name of vessel.....
Date of shipment.....
Port of landing in England or Wales.

THIRD SCHEDULE.

This is to certify that the Colorado Beetle (*Leptinotarsa decemlineata*) does not exist and has not been known to exist within a radius of at least 200 kilometres of the place where the living plants, raw vegetables, or cider apples included in the package or consignment described below were grown.

(Signed).....

(Official status).....

No. and description of packages.....
Distinguishing marks.....
Description of living plants, raw vegetables or cider apples .....
.....
Stated to be grown at.....
Exported by.....
Name and address of consignee.....
.....
Name of vessel.....
Date of shipment.....
Port of landing in England or Wales.....

FOURTH SCHEDULE.

This is to certify that the raw apples included in the package or consignment described below are of one of the following grades as recognised by the Department of Agriculture of the United States of America.

"U. S. Fancy." "Extra Fancy."
"U. S. (No. 1)." "Fancy."

Signature.....
Official status.....
Date.....

Description of Consignment.

Number and Nature of Packages.....
Distinguishing marks.....
.....
Variety of Apples.....
Name and address of consignee .....
.....
Name of vessel.....
Date of shipment.....
Port of shipment.....

FIFTH SCHEDULE.

Colorado Beetle (Customs) Order of 1877. (*a*)
Destructive Insects and Pests Order of 1922. (*b*)
Destructive Insects and Pests (Amendment) Order of 1927. (*c*)
Colorado Beetle Order of 1924. (*d*)
Colorado Beetle Order of 1925. (*e*)
Colorado Beetle Order of 1931. (*f*)
Importation of Potatoes (Canary Islands) Order of 1927. (*g*)
Importation of Potatoes (Malta) Order of 1927. (*h*)
Importation of Raw Apples Order of 1930. (*i*)

(*a*) S. R. & O. Rev. 1904, IV, Destructive Insect, p. 1.
(*b*) S. R. & O. 1922 (No. 583), p. 178.
(*c*) S. R. & O. 1927 (No. 1129). p. 339.
(*d*) S. R. & O. 1924 (No. 1470), p. 236.
(*e*) S. R. & O. 1925 (No. 174), p. 241.
(*f*) S. R. & O. 1931 (No. 879), p. 259.
(*g*) S. R. & O. 1927 (No. 377), p. 336.
(*h*) S. R. & O. 1927 (No. 571), p. 338.
(*i*) S. R. & O. 1930 (No. 522), p. 440.

Errata to Volume III, Part V.

Plate LXIX, Figure 4 (letter-press) *for* " abundance " *read* " absence ".

Page 862, para. (*b*), line 8, *delete* " on ".

Page 863, line 9, *for* " prussic acid *read* " potassium cyanide ".

ORIGINAL ARTICLES

STUDIES ON *PLATYEDRA GOSSYPIELLA* SAUNDERS, THE PINK BOLLWORM OF COTTON, IN THE PUNJAB.

PART III. PHOTOTROPIC RESPONSE OF *P. GOSSYPIELLA*.

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(With four text-figures)

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I. INTRODUCTION.

Divergent views have been expressed regarding the phototropic response of *P. gossypiella* Saund. Stuhlmann [1907] working in the former German East

Africa (Tanganyika Territory), Busck [1917] in Hawii, and Loftin *et al* [1921] in Mexico, maintain that the moths are *not* positively phototropic. In fact, Busck even goes so far as to say that those who declare that this moth is attracted to artificial light have probably wrongly identified the specimens collected. On the contrary, Lefroy [1906] in India, Willcocks [1916], Gough [1919] and Ballou [1920] in Egypt, Kanbe [1928] in Korea, and Delassus [1931] in Algeria have positive evidence that the moths are strongly attracted to artificial light. Our observations during the four years (1929-1932) have established, without a shadow of doubt, that in the Punjab *P. gossypiella* moths are positively phototropic. We have collected moths at light traps in the field, and have made use of the phototropic response in various other experiments. Evidently, therefore, it is a case of the same insect behaving differently in different countries, or, in other words, under different environmental conditions.

Observations and experiments on phototropism recorded in this paper were carried out during the three years, 1929-1931, at Rohtak, which is situated in the South-Eastern region of the Punjab—a region that shows the highest intensity of Pink Bollworm attack in this province. The light trap was mounted on a stand six feet high, *i.e.*, well above the cotton crop. A 'Petromax' incandescent gas lamp of two hundred candle power was the source of light. The trap was set at sunset and removed at sunrise. Observations were started on July 16th in all the three years, and were continued up to the end of November during 1929 and 1930, and up to the first week of November in 1931.

This work was carried out as a part of the Punjab Entomological Scheme financed by the Indian Central Cotton Committee. Our thanks are due to Mr. Mohammad Afzal, Cotton Research Botanist, Lyallpur, for his constant help and criticism.

II. Seasonal history of the cotton crop and *P. gossypiella* at Rohtak.

The varieties of cotton sown at Rohtak are mostly strains of *Gossypium indicum* var. *Mollisoni*. Sowing is done from the beginning of April to the middle of May, the bolls start appearing from the beginning of August, and picking starts from about the middle of September, and is almost over by the first week of November. In 1929 and 1930 the bolling period was normal, and the first picking was done on 8th and 14th September respectively. During 1931, however, the crop was very late, and picking started from the 1st of October.

In the Punjab *P. gossypiella* has two distinct types of life-cycles, the 'short-cycle', where the full-grown caterpillar pupates immediately, and the 'long-cycle', where the full-grown caterpillar passes through many months of 'hibernation' in the cotton seeds or, rarely, in lint. Hibernation sets in by

about the beginning of October and this coincides with the maturing of the crop. By the end of the month almost all the caterpillars found are 'long-cycle'. Moths begin to emerge in small numbers from the hibernating 'long-cycle' larvae from the beginning of April, but by far the largest number emerges during the rains, *i.e.*, in July and early part of August. The progeny of those moths which emerge up to the end of June die on account of lack of food [Afzal Husain *et al* 1931], and it is only the progeny of moths emerging after this date that is mainly responsible for starting the attack on the new cotton crop. The moths of the first brood of the season appear from about the middle of August, and there are in all three or four broods during a cotton season. The population of the moths is highest from about the middle of September to the middle of October after which it begins to fall.

III. Moths trapped.

The details of the daily captures of moths together with maximum and minimum temperatures, rainfall, lunar dates and picking dates are given in Table I. All these data, except the temperature records, are represented graphically in Figs. 1-3. Pink Bollworm moths started coming to the light trap from 16th July in 1929 and 1930, although in very small numbers. In 1931, however, the first moth was trapped on 3rd August, *i.e.*, 18 days later. During this year, as stated above, the crop was late by about a fortnight. Again, during the first two years a fair number of moths was trapped during August, 193 moths in 1929 and 369 in 1930, but in August 1931 exceedingly few moths, only 9, were trapped, evidently because very few were present. During September the number of moths attracted was the highest in the first two years, 2297 in 1929, and 1186 in 1930, but in 1931 the number attracted was once again very small—only 260. In October 1931, however, a very large number of moths, 2815, was attracted whereas much smaller numbers 1336 and 864, had been attracted in the same month during 1929 and 1930.

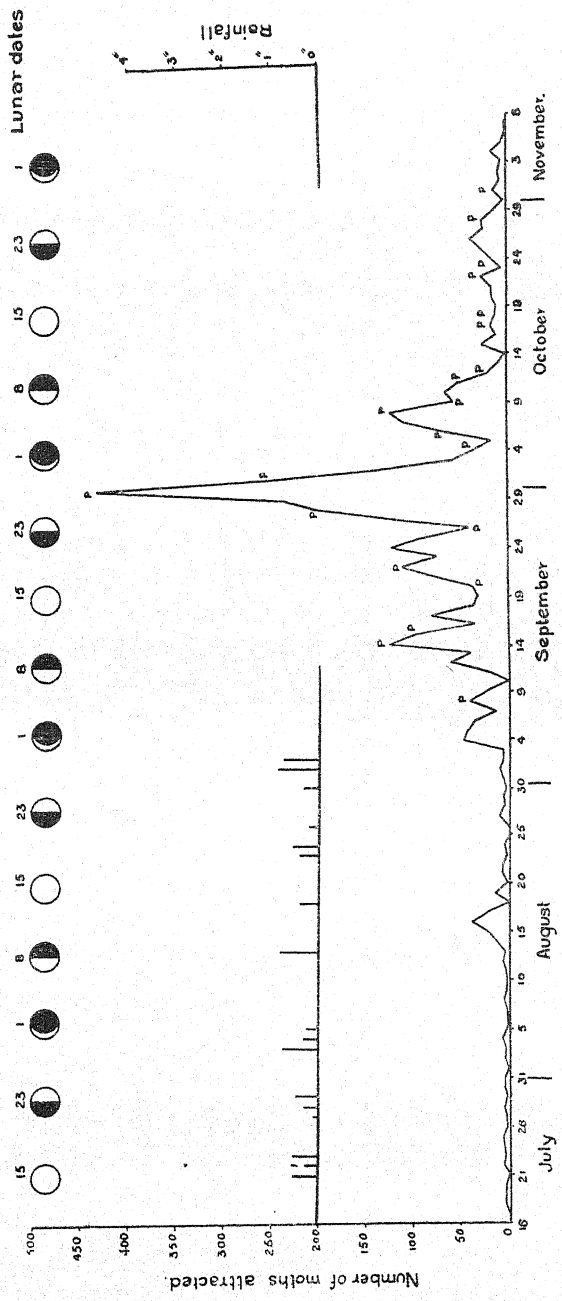


Fig. 1.—Daily capture of moths during 1929.

P-date of picking.

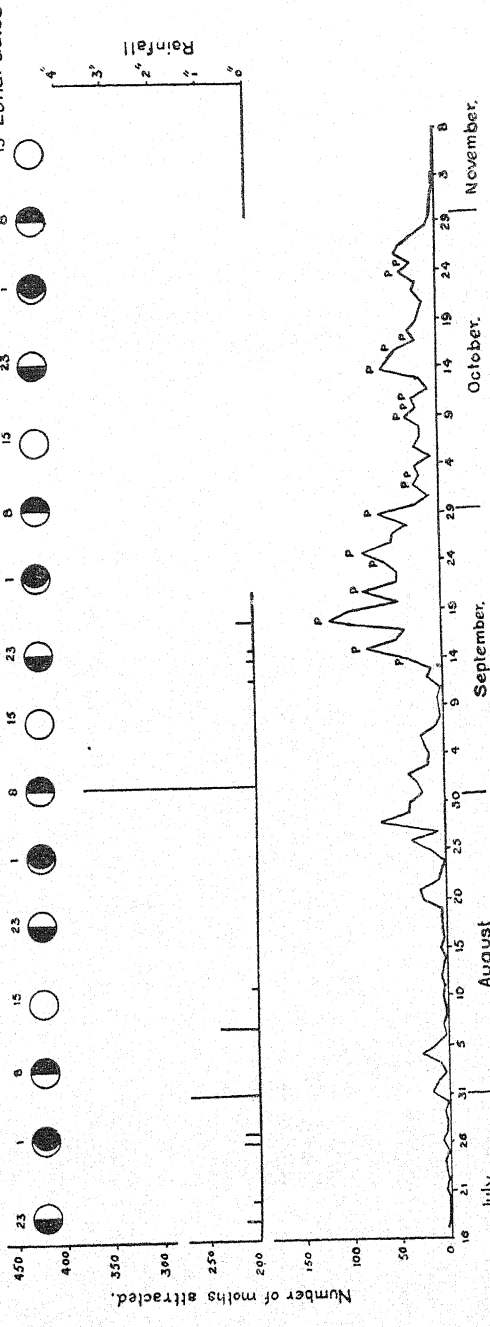


Fig. 2.—Daily capture of moths during 1930.

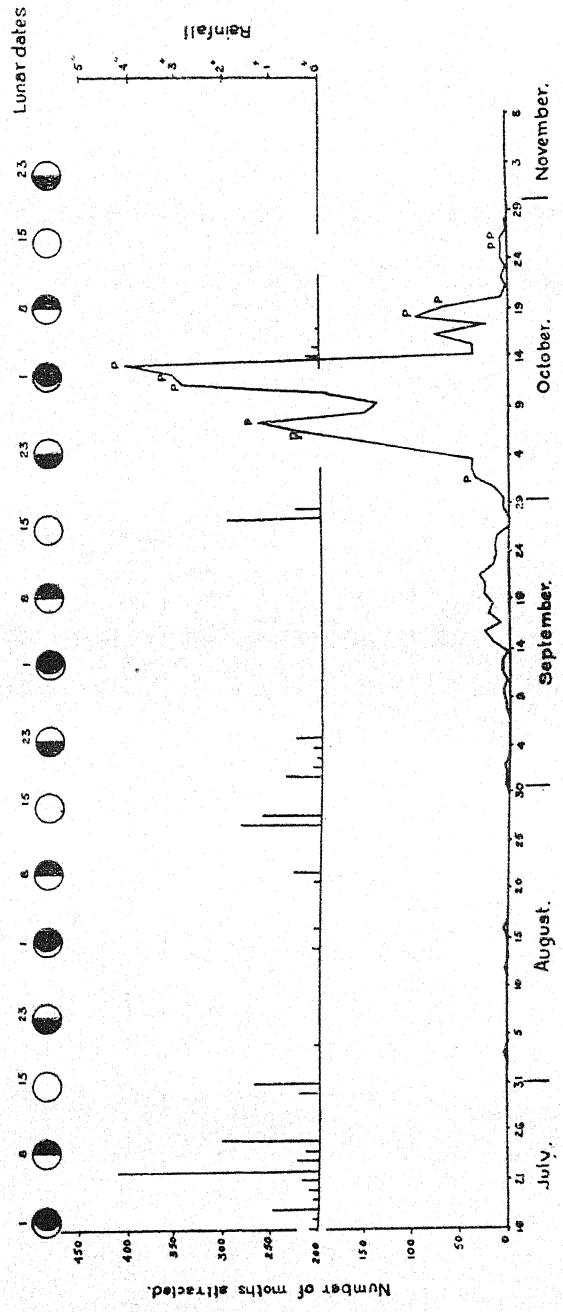


Fig. 3.—Daily capture of moths during 1931.

TABLE I.

Daily attraction of P. gossypiella *moths and the meteorological data 1929-1931.*

Date	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)
	1929					1930					1931				
July.															
16 . .	103	88	1	9	..	90	80	1	20	1·47	93	84	0	30	0·16
17 . .	102	88	0	10	..	92	79	1	21	..	92	82	0	1	0·07
18 . .	102	82	3	11	..	94	80	0	22	0·22	91	80	0	2	0·99
19 . .	100	84	4	12	..	94	82	0	23	..	91	82	0	3	..
20 . .	96	80	3	13	..	97	86	1	24	0·15	97	80	0	4	0·10
21 . .	86	81	0	14	0·36	98	84	1	25	..	86	76	0	5	0·28
22 . .	86	81	5	15	0·57	94	82	0	26	..	88	73	0	6	4·20
23 . .	90	80	3	16	0·46	98	82	1	27	..	96	80	0	7	0·41
24 . .	90	82	3	17	..	97	84	3	28	..	96	76	0	8	0·25
25 . .	96	82	5	18	..	94	82	1	29	..	94	83	0	9	1·96
26 . .	97	82	2	19	..	93	82	4	30	0·25	98	85	0	10	..
27 . .	96	81	2	20	0·1	94	82	2	1	0·30	97	85	0	11	..
28 . .	89	78	4	21	0·3	94	82	0	2	..	102	82	0	12	..
29 . .	96	82	2	22	0·44	97	82	1	3	..	102	78	0	13	..
30 . .	94	82	3	23	..	93	78	0	4	..	95	76	0	14	0·41
31 . .	96	82	3	24	..	90	80	17	5	1·52	94	83	0	15	1·30
August.															
1 . .	96	83	1	25	..	94	82	10	6	..	97	87	0	16	..

TABLE I—*contd.*

Daily attraction of P. gossypiella *moths and the meteorological data 1929-1931*—contd.

Date	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)
	1929					1930					1931				
August—contd.															
2 . .	98	82	2	26	..	95	82	4	7	..	95	86	0	17	..
3 . .	94	80	3	27	0·77	95	82	8	8	..	98	81	1	18	..
4 . .	93	81	5	28	0·33	98	82	25	9	..	93	81	0	19	0·06
5 . .	96	82	2	29	0·24	94	82	11	10	..	97	83	1	20	..
6 . .	97	82	1	1	..	97	82	2	11	..	99	84	0	21	..
7 . .	97	82	2	2	..	96	82	4	12	0·79	91	81	0	22	..
8 . .	96	82	4	3	..	94	82	2	13	..	100	84	0	23	..
9 . .	95	83	3	4	..	94	82	2	14	..	100	84	0	24	..
10 . .	100	82	1	5	..	86	80	4	15	..	95	84	0	25	..
11 . .	101	83	2	6	..	90	78	2	16	0·1	98	84	1	26	..
12 . .	102	84	6	7	..	94	82	3	17	..	99	86	2	27	..
13 . .	101	84	5	8	0·8	97	80	2	18	..	98	86	0	28	..
14 . .	99	82	11	9	..	98	78	1	19	..	99	86	1	29	0·04
15 . .	90	79	21	10	..	98	80	3	20	..	98	82	1	1	..
16 . .	94	82	38	11	..	101	82	2	21	..	99	86	2	2	0·08
17 . .	92	80	23	12	..	103	83	2	22	..	99	84	0	3	..
18 . .	89	80	0	13	0·4	98	80	4	23	..	98	85	0	4	..
19 . .	90	80	14	14	..	100	80	3	24	..	100	84	0	5	..
20 . .	97	80	2	15	..	98	80	21	25	..	95	84	0	6	..
21 . .	96	82	6	16	..	102	78	24	26	..	95	84	0	7	0·13
22 . .	92	80	5	17	..	103	81	7	27	..	96	84	0	8	0·52

23 . .	92	79	2	18	0·4	102	82	3	28	..	98	84	0	9	..
24 . .	97	80	3	19	0·55	103	83	1	29	..	98	85	0	10	..
25 . .	94	80	0	20	..	102	82	12	1	..	99	88	0	11	..
26 . .	89	76	0	21	0·21	103	82	35	2	..	98	82	0	12	..
27 . .	85	76	10	22	..	103	84	6	3	..	95	82	0	13	1·64
28 . .	86	78	6	23	..	102	80	68	4	..	94	83	0	14	1·17
29 . .	89	77	5	24	0·25	105	83	38	5	..	97	84	0	15	..
30 . .	88	77	3	25	0·01	100	82	37	6	..	94	84	0	16	..
31 . .	94	78	7	26	0·83	100	76	23	7	..	95	81	0	17	..
September.															
1 . .	97	80	10	27	0·64	96	78	26	8	3·84	96	82	0	18	0·70
2 . .	96	78	6	28	..	98	75	36	9	..	96	82	0	19	0·17
3 . .	96	78	6	29	..	103	78	17	10	..	94	78	0	20	0·05
4 . .	98	78	49	30	..	103	78	15	11	..	93	80	0	21	0·15
5 . .	100	77	43	1	..	103	76	18	12	..	94	79	0	22	0·45
6 . .	102	77	36	2	..	104	77	21	13	..	88	78	0	23	..
7 . .	102	82	14	3	..	105	80	6	14	..	94	76	0	24	..
8 . .	102	82	41P*	4	..	107	82	2	15	..	94	76	3	25	..
9 . .	98	78	26P	5	..	108	84	3	16	..	95	76	4	26	..
10 . .	99	78	0	6	..	107	83	4	17	..	97	77	4	27	..
11 . .	98	77	20	7	..	101	82	2	18	..	97	76	3	28	..
12 . .	98	72	60	8	..	98	79	15	19	0·04	98	74	5	29	..
13 . .	99	74	41	9	..	95	76	11	20	..	97	74	7	1	..
14 . .	102	74	125 P	10	..	94	76	35 P	21	0·08	98	78	4	2	..
15 . .	102	75	97 P	11	..	94	78	76 P	22	0·02X†	99	75	16	3	..
16 . .	104	78	36	12	..	98	78	48	23	..	100	77	25	4	..
17 . .	105	72	81	13	..	102	78	40	24	..	102	76	10	5	..
18 . .	102	70	38	14	..	102	77	119 P	25	0·18 X	102	78	22	6	..
19 . .	105	73	34	15	..	103	75	97	26	..	101	76	19	7	..
20 . .	106	72	38 P	16	..	104	71	45	27	..	99	79	23	8	—

* P indicates the picking dates.

† X indicates nights on which 4-hourly collections weremade.

TABLE I—*contd.*

Daily attraction of P. gossypiella *moths and the meteorological data 1929-1931*—contd.

Date	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)
	1929					1930					1931				
September.															
21 . .	106	71	77 P	17	..	101	68	81 P	28	..	100	80	23	9	..
22 . .	106	72	112 P	18	..	101	68	48	29	..	101	80	31	10	..
23 . .	106	74	65	19	..	102	68	47	1	X	98	79	14	11	X
24 . .	106	74	124	20	..	102	70	59 P	2	..	94	80	12	12	..
25 . .	106	74	92	21	..	104	71	81 P	3	..	88	78	13	13	..
26 . .	102	76	41 P	22	..	105	69	53	4	..	92	79	11	14	..
27 . .	104	79	120 P	23	..	104	68	50	5	X	94	76	0	15	..
28 . .	101	79	201	24	..	102	70	36	6	..	90	76	0	16	1·98
29 . .	102	80	235	25	..	103	68	74 P	7	..	90	77	5	17	0·45
30 . .	102	82	429 P	26	X	105	66	21	8	..	93	79	6	18	X
October.															
1 . .	104	79	257 P	27	X	104	62	11	9	..	99	82	15 P	19	..
2 . .	98	76	140	28	0·09 X	106	62	28 P	10	..	94	78	34	20	..
3 . .	92	72	58	29	..	105	64	21 P	11	..	94	77	38	21	..
4 . .	88	70	39 P	1	..	106	64	25	12	..	94	76	37	22	X
5 . .	94	68	19 P	2	X	106	64	8	13	..	94	76	98	23	..
6 . .	98	69	63	3	X	106	66	26	14	..	94	76	157	24	X
7 . .	100	74	109	4	X	104	66	18	15	..	95	76	217 P	25	..
8 . .	98	74	124 P	5	..	102	68	19	16	..	96	78	261 P	26	X
9 . .	98	70	57 P	6	X	103	70	34 P	17	..	97	80	151	27	..

10 . .	98	68	64	7	..	106	69	23 P	18	X	98	80	138	28	..
11 . .	99	66	52	8	..	104	73	26 P	19	..	98	76	190	29	X
12 . .	100	68	21 P	9	..	106	72	10	20	..	100	78	342 P	30	X
13 . .	99	67	10 P	10	..	106	74	19	21	..	99	77	355 P	1	..
14 . .	100	66	3	11	..	102	70	60 P	22	X	93	76	402 P	2	..
15 . .	100	68	29	12	..	104	60	52	23	..	86	74	38	3	0·24
16 . .	99	69	12	13	..	104	77	45 P	24	..	87	72	39	4	0·10
17 . .	99	70	18 P	14	..	90	67	21 P	25	X	94	72	77	5	..
18 . .	96	58	14 P	15	X	98	66	30	26	..	93	65	25	6	0·04 X
19 . .	94	58	12	16	X	102	70	22	27	..	92	61	96 P	7	..
20 . .	92	58	16	17	..	102	65	19	28	..	90	59	65 P	8	X
21 . .	95	58	18	18	..	94	60	14	29	..	91	61	6	9	..
22 . .	98	68	20 P	19	X	98	72	24	30	..	93	60	3	10	..
23 . .	99	74	7 P	20	..	98	66	21	1	X	91	56	6	11	..
24 . .	98	64	19	21	..	98	66	40 P	2	..	89	58	3	12	..
25 . .	94	56	28	22	..	95	63	25 P	3	X	91	58	6	13	X
26 . .	93	56	39	23	..	95	64	43	4	..	92	56	7 P	14	..
27 . .	94	55	26 P	24	..	90	62	37	5	..	92	59	7 P	15	X
28 . .	96	55	24 P	25	X	92	69	24	6	..	90	60	3	16	..
29 . .	98	54	13	26	..	85	70	9	7	X	91	68	2	17	..
30 . .	94	53	3	27	..	76	58	5	8	..	83	62	0	18	..
31 . .	94	55	13 P	28	..	87	56	5	9	X	88	55	0	19	..
November.															
1 . .	96	55	8	29	..	90	51	3	10	..	89	52	0	20	..
2 . .	95	58	9	30	..	89	51	2	11	..	87	52	0	21	..
3 . .	96	58	7	1	..	89	52	2	12	..	89	53	0	22	..
4 . .	90	50	15	2	..	88	50	0	13	..	90	82	0	23	..
5 . .	88	51	6	3	..	86	46	0	14	..	87	50	0	24	..

TABLE I—*contd.*

Daily attraction of P. gossypiella *moths and the meteorological data 1929-1931*—concld.

Date	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)	Daily max. temp. (°F.)	Daily min. temp. (°F.)	No. of moths captured	Lunar date	Rain-fall (inches)
	1929					1930					1931				
November															
6 . .	89	47	1	4	..	86	46	0	15	..	..	..	..	..	..
7 . .	90	46	0	5	..	90	46	0	16	..	..	..	..	..	..
8 . .	90	50	0	6	..	..	..	..	..	..	..	..	..	..	..
9 . .	88	56	0	7	..	..	..	..	..	..	..	..	..	..	..
10 . .	90	51	0	8	..	..	..	..	..	..	..	..	..	..	..

The monthly catches of Pink Bollworm moths, their percentages, and the total number caught during individual years are given in Table II. It will be noticed that the largest number of moths was caught in 1929 and the smallest number in 1930, but, in spite of this difference, there is a very close similarity in the catches of moths during various months. During 1931, the period of attraction was very short as compared with the two previous years and a very large percentage of moths, over 90 per cent., was trapped in October, and none at all in July and November.

TABLE II.

Monthly totals of the attraction of P. gossypiella *moths at Rohtak.*

Year		July	August	September	October	November	Total
1929	Actual . .	43	193	2297	1336	46	3915
	Percentage . .	1·1	4·9	58·7	34·1	1·2	
1930	Actual . .	33	369	1186	864	7	2459
	Percentage . .	1·3	15·0	48·2	35·1	0·4	
1931	Actual . .	0	9	260	2815	0	3084
	Percentage . .	0	0·3	8·4	91·3	0	

The very low attraction of moths in the beginning in 1931 and the very large attraction later in the season, *i.e.*, in October, shows that the increase in *P. gossypiella* population is connected with the development of the crop. The crop was late this year and the attack of the pest, as will be shown elsewhere, was also late in developing. Williams' [1926] conclusion, that the development of Pink Bollworm attack is directly limited by the state of development of the cotton crop, receives corroboration from our catches of Pink Bollworm moths in 1931.

Another point that will be noticed from Table II is that, in each of the three years, over 80 per cent. of the moths were attracted in September and October, and very few during the rest of the period. This, in the light of what has been said about the seasonal history of the pest, is what should be expected. During July and August very few moths were trapped because at this time the population of Pink Bollworm moths was very small. By September and first half of October the pest had multiplied considerably, and the largest number of the moths was attracted. Finally with the setting in of hibernation the population of moths

began to fall from about the middle of October, and no moth was attracted after the first week of November.

IV. Hours of the night most favourable for the attraction of *P. gossypiella*.

Of the workers who have studied the phototropic response of Pink Bollworm moths, only Ballou [1920] in Egypt and Delassus [1931] in Algeria have taken into account the time at which these moths are attracted to light. While Ballou arrives at the conclusion that these moths are dusk fliers, and that their activity is greatest during the short period after sun-set, Delassus observes that the numbers captured are often greater at dawn than in the evening.

On certain nights, during all the three years, 1929-1931, four-hourly collections were made and the number of moths captured during each of these periods was determined (Table III). It will be seen that in 1929 the maximum attraction on 30th September, 1st and 2nd October was noticed during the last four hours of the night (2-6) and the intermediate four-hour period (22-2) came next. From 5th October to 22nd October, on the other hand, the largest number was attracted during the intermediate four-hour period, while from 28th October the first four-hour period (18-22) was the most favourable.

TABLE III.

Attraction of P. gossypiella *moths to light during three different four-hour periods of the night.*

1929							1930							1931						
Date	No. of moths captured during different hours of the night			Range of temperature (°F.) during different hours of the night			Date	No. of moths captured during different hours of the night			Range of temperature (°F.) during different hours of the night			Date	No. of moths captured during different hours of the night			Range of temperature (°F.) during different hours of the night		
	Percentage							Percentage							Percentage					
	18-22	22-2	2-6	18-22	22-2	2-6		18-22	22-2	2-6	18-22	22-2	2-6		18-22	22-2	2-6	18-22	22-2	2-6
Sept.							Sept.							Sept.						
30	73/17	126/30	230/53	99-89	89-84	84-82	2	3/12	22/88	0/0	93-89	89-78	78-75	20	1/4	7/28	17/68	99-86	86-82	82-79
Oct.							6	4/22	12/67	2/11	95-88	88-79	79-77	23	0/0	4/29	10/71	92-86	86-80	80-79
1	62/24	72/29	123/47	98-88	88-83	83-79	12	3/19	11/69	2/12	83-80	80-79	80-79	30	2/33	1/17	3/50	94-85	85-81	81-79
2	7/5	59/40	74/55	90-84	84-76	76-76	15	30/39	35/46	11/15	90-84	84-79	79-78	Oct.						
5	7/37	7/37	5/26	91-77	77-71	71-68	18	49/41	50/42	20/17	95-87	87-79	79-77	4	13/35	20/54	4/11	92-84	84-79	79-76
6	23/37	28/44	12/19	93-80	80-74	74-69	23	20/43	23/48	4/9	90-75	75-69	69-68	6	20/13	118/75	19/12	92-84	84-80	80-76
7	5/5	81/74	23/21	95-84	84-76	76-74	26	11/21	40/75	2/4	92-79	79-72	72-69	8	33/21	122/75	6/4	92-85	85-83	83-78
9	11/19	29/51	17/30	94-86	86-73	73-70	29	3/4	57/77	14/19	89-78	78-70	70-68	11	59/31	78/41	53/28	92-83	83-78	78-76
18	4/29	9/64	1/7	91-71	71-66	66-58	Oct.							12	127/37	148/46	57/17	95-86	86-80	80-78
19	6/50	6/50	0/0	90-71	71-62	62-58	10	9/39	10/44	4/17	95-78	78-72	72-69	18	27/54	20/40	3/6	86-75	75-68	68-65
22	8/28	14/48	7/24	92-77	77-71	71-68	14	11/57	6/32	2/11	95-80	80-75	75-70	20	29/58	16/32	5/10	83-69	69-61	61-59
28	11/46	9/37	4/17	91-70	70-63	63-55	17	5/24	16/76	0/0	85-78	78-69	69-67	25	5/83	1/17	0/0	79-67	67-60	60-58
							22	4/17	16/66	4/17	91-79	79-78	78-72	27	5/71	2/29	0/0	88-72	72-66	66-59
							25	1/4	24/96	0/0	88-76	76-68	68-63							
							29	7/78	2/22	0/0	79-74	74-70	70-70							
							31	5/100	0/0	0/0	79-67	67-59	59-56							

In 1930, during September and up to the 25th October, the maximum number of moths, with one exception of 14th October, was attracted in the intermediate four-hour period and the first four-hour period came next, while from 29th October the largest number of moths was attracted during the first four-hour period and none in the last four-hour period.

In 1931 the attraction was similar to that of 1929 and the maximum number of moths, from 20th September to 30th September, was captured during the last four-hour period, while from 4th October to 12th October the largest number was collected during the intermediate four-hour period, and from 18th to 27th October during the first four-hour period.

It appears from the data presented that earlier in the season the largest number of moths was attracted during the last four-hour period, and later on during the intermediate four-hour period and finally, at the end of the season, *i.e.*, from about the last week of October onwards, the majority of the moths were attracted during the first four-hour period.

Another point that will be noticed from Table III is that, with few exceptions, the moths came to light throughout the night.

V. Effect of temperature on the phototropic response of *P. gossypiella*.

The direct influence of temperature on the phototropic response of insects has been studied by various workers. Chapman [1923] found that the attraction of *Taphrocerus gracialis* to light depends entirely on temperature. At 30°C. (86°F.) the beetles are very active and respond positively to light, but at 15°C. (59°F.) their response to light becomes negative. Fraenkel, Bodenheimer and others [1930] studied the problem with regard to the hoppers of the desert locust and found that they were positively phototropic when the temperature of the air and their body was 20°C. (68°F.), while at 30°C.-35°C. (86°-95°F.) they became indifferent to light. Yothers [1926] also found a very close correlation between the average daily temperature and the codling moth catches in trap-baits. Similarly Cook [1921], who studied the influence of all weather factors on the capture of nocturnal Lepidoptera, arrived at the conclusion that temperature had a positive effect on the captures, its influence being much larger below than above optimum humidity.

Laboratory experiment.—To study the influence of temperature on the attraction of *P. gossypiella* to light, the following apparatus was designed. Two cylinders, A and B, were placed one inside the other. The inner cylinder A had two holes, to one was attached a glass tube and the other contained a thermometer. Tempera-

ture in the inner cylinder was controlled by circulating water in the outer cylinder B. The latter was placed in a box packed with saw-dust and the temperature in A was kept constant.

Moths were placed in the inner chamber and kept for about 15 minutes, to allow them time to acquire the temperature of the surrounding air, and then the light was put up. The moths were attracted in the tube. Results of these observations are given in Table IV. At 50°F. no moths were attracted into the tube. At 59°F. the number attracted was very small, the average being 5 per cent. and the highest 12·0 per cent. At 68°F. attraction ranged from 20 to 37 per cent. with an average of 29 per cent., while at 77°F. the number of moths attracted was the highest, varying from 30 to 70 per cent., the average being 55 per cent. At 86°F. there was a fall and the attraction ranged from 30 to 57 per cent. with an average of 45 per cent. and at 95°F. it was from 20 to 50 per cent. with an average of 27·3 per cent. (Fig. 4).

Exposure to 104°F. and 113°F. showed a very interesting phenomenon. The moths exposed for 15 minutes to 104°F. showed low attraction, ranging from 25 to 34 per cent. but when the time of exposure was increased to 20 minutes no moth was attracted. Similar exposures of 15 and 20 minutes to 113°F. gave negative results. However, when the time of exposure at both these temperatures was reduced to 5 minutes the proportion of moths attracted was very large; at 104°F. 64 to 93 per cent. moths were attracted, and at 113°F. 86 to 92 per cent.

TABLE IV.

Attraction of P. gossypiella *moths to light under controlled conditions.*

Date	Temp. (°F.) to which exposed	Duration of exposure	No. of moths introduced	No. of moths attracted	Percentage attraction
8th October 1930 .	50	15 mins.	50	0	0
13th „ 1931 .	50	„	70	0	0
17th „ „ .	50	„	50	0	0
24th „ „ .	50	„	30	0	0
Total .	..	..	200	0	Average: 0

TABLE IV—(*contd.*)

Date	Temp. (°F.) to which exposed	Duration of exposure	No. of moths introduced	No. of moths attracted	Percentage attraction
9th October 1930	59	15 mins.	20	0	0
10th ,, ,,	59	,,	20	0	0
25th ,, ,,	59	,,	10	0	0
28th ,, ,,	59	,,	18	1	5·5
13th ,, 1931	59	,,	70	2	2·9
17th ,, ,,	59	,,	50	6	12·0
24th ,, ,,	59	,,	30	2	6·6
Total	..	..	218	11	Average: 5·04
11th October 1930	68	15 mins.	25	7	28·0
15th ,, ,,	68	,,	25	6	24·0
17th ,, ,,	68	,,	15	5	33·3
13th ,, 1931	68	,,	70	26	37·1
17th ,, ,,	68	,,	50	14	28·0
24th ,, ,,	68	,,	30	6	20·0
Total	..	..	215	64	Average: 29·7
12th October 1930	77	15 mins.	45	27	60·0
29th ,, ,,	77	,,	10	7	70·0
12th ,, 1931	77	,,	50	29	58·0
15th ,, ,,	77	,,	50	29	58·0
27th ,, ,,	77	,,	20	9	45·0
29th ,, ,,	77	,,	15	6	40·0
7th November ,,	77	,,	10	3	30·0
Total	..	..	200	110	Average: 55·0

TABLE IV—*contd.*

Date	Temp. (°F.) to which exposed	Duration of exposure	No. of moths introduced	No. of moths attracted	Percentage attraction
13th October 1930 .	86	15 mins.	20	9	45·0
14th ,, ,, .	86	,,	30	17	56·7
12th ,, 1931 .	86	,,	45	21	46·6
15th ,, ,, .	86	,,	50	23	46·0
27th ,, ,, .	86	,,	20	6	30·0
28th ,, ,, .	86	,,	15	7	46·7
29th ,, ,, .	86	,,	15	6	40·0
7th November ,,	86	,,	10	4	40·0
Total .	..	..	205	93	Average: 45·3
16th October 1930 .	95	15 mins.	10	5	50·0
15th ,, 1931 .	95	,,	50	14	28·0
27th ,, ,, .	95	,,	20	5	25·0
28th ,, ,, .	95	,,	15	3	20·0
29th ,, ,, .	95	,,	15	3	20·0
Total .	..	..	110	30	Average: 27·3
23rd October 1930 .	104	15 mins.	12	3	25·0
24th ,, ,, .	104	,,	15	5	33·3
11th August 1932 .	104	,,	50	17	34·0
Total .	..	..	77	25	Average: 32·5
25th October 1931 .	104	20 mins.	35	0	0
Total .	..	..	35	0	Average: 0

TABLE IV—*concld.*

Date	Temp. (°F.) to which exposed	Duration of exposure	No. of moths introduced	No. of moths attracted	Percentage attraction
22nd October 1930 .	113	15 mins.	15	0	0
„ „ „ .	113	20 „	10	0	0
18th „ 1931 .	113	15 „	30	0	0
19th „ „ .	113	15 „	50	0	0
Total .	..	..	105	0	Average: 0
11th October 1930 .	104	5 mins.	22	14	63·7
18th „ 1931 .	104	„	50	43	86·0
1st November „ .	104	„	15	14	93·3
8th August 1932 .	104	„	50	44	88·0
Total	..	..	137	115	Average: 83·9
20th October 1930 .	113	5 mins.	15	13	86·6
12th August 1932 .	113	„	50	46	92·0
Total .	..	..	65	59	Average: 90·8

There can be little doubt that the high attraction of moths after such short exposures was in the nature of escape rather than phototropic response, and that longer exposures probably made most of the moths inactive and indifferent to light.

Field data.—To compare the results of attraction at constant temperatures with those in the field, as stated above, four-hourly collections were made on certain

nights, and temperature was recorded by placing a thermograph in an adjoining field (Table III).

A correlation between the number of moths collected and the precise degree of temperature is difficult, because temperature within a four-hour period varied, and there was variation from day to day for the same period. Thus it is difficult to say what temperature was most favourable. It has, however, been assumed (quite arbitrarily) that during a particular four-hour period, each degree of temperature was responsible for attracting an equal number of moths. It is evident that this procedure reduces the value of more favourable temperatures and increases the value of the temperatures slightly less favourable. However, as will be shown later, this is the best method, and by dividing the number of moths attracted during a particular four-hour period by the degrees of temperature, the number of moths attracted to a particular temperature has been ascertained. This process has been followed with all the four-hour periods of different nights, and all the moths attracted at different temperatures have been added up.

The data thus collected have been classified by grouping three successive degrees of temperature and adding up the moths attracted in each group. These are plotted on the graph to represent the frequencies of various groups of three-degree temperatures. Such absolute histograms have been prepared for all the three years in which observations were carried out (1929-1931). To sum up the results of the three years the number of moths corresponding to the various three-degree groups for all the three years have been added up, and a percentage histogram prepared from the totals. Here grand total is considered as 100 and the percentages for the various groups have been deduced therefrom. This percentage histogram has been further smoothed.

The method adopted above is purely arbitrary but seems to answer the purpose. Mean temperature of the four-hour period could not have been taken, as that would not represent the temperature prevailing in any period, for any length of time; nor could *mode* (that temperature which prevailed for most of the time) be taken because it cannot be said that this temperature alone was responsible for the number of moths attracted.

Fig. 4 is a graphic representation of the data so collected, and shows that the optimum zone for the attraction of moths ranges from 76°F. to 87°F. This optimum zone corresponds very closely with the results obtained under controlled conditions of temperature, where, as has been shown already, the maximum number of moths was attracted at 77° and 86°F.

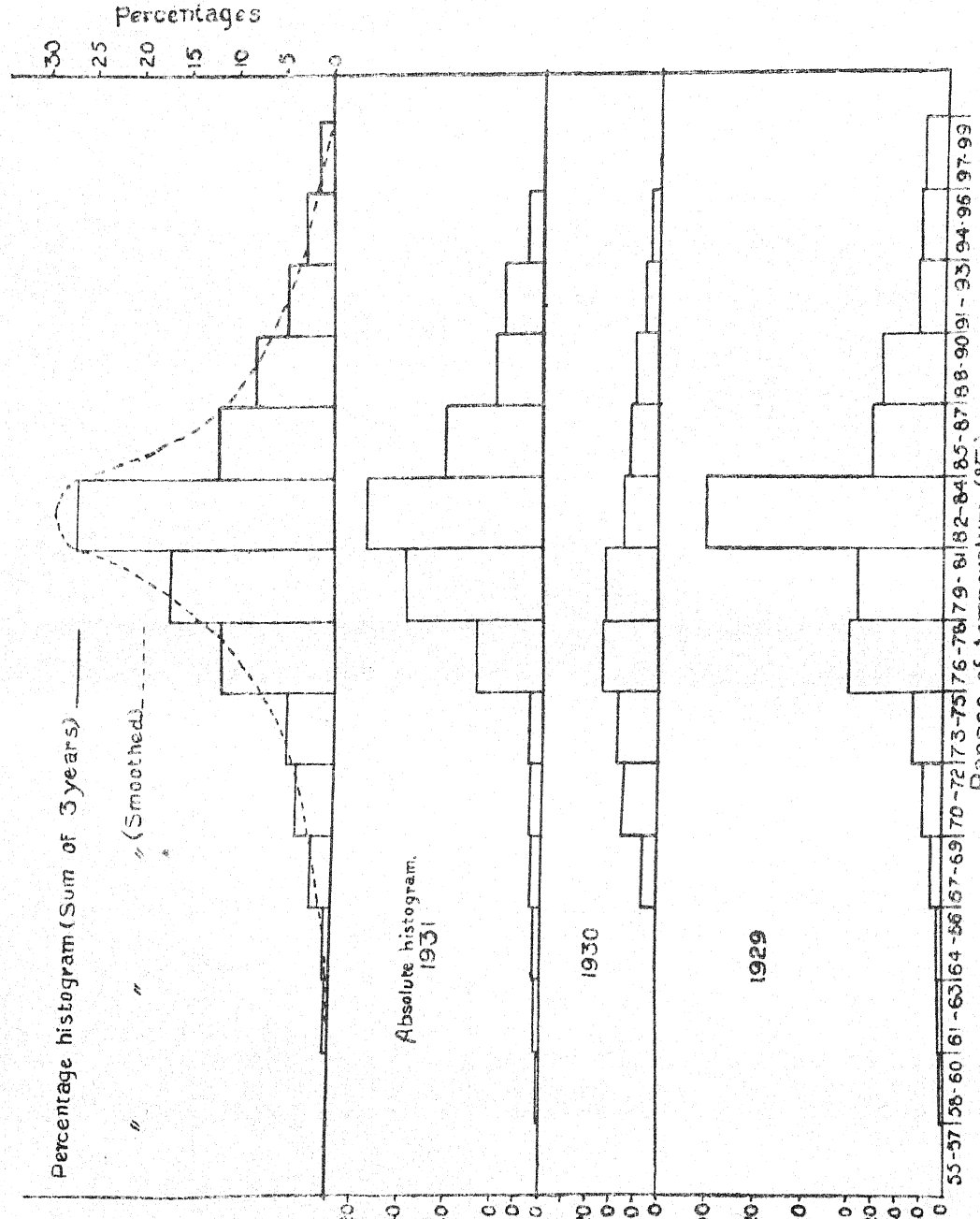


Fig. 4.—Effect of temperature on the daily capture of moths.

From the four-hourly collections made on certain nights it will be seen that in the majority of cases the largest number of moths was attracted during those four-hour periods when the temperature was within or near the optimum range. Thus during 1929, on 30th September and 1st October the optimum temperature ranged during the last four-hour period when the maximum attraction took place; later the optimum temperature ranged during intermediate and first four-hour periods respectively and the largest number of moths was also attracted at that time.

In 1930, on nights when four-hourly collections were made, optimum temperature did not usually prevail in the last four-hour period, and we find that the maximum attraction took place either in the first or in the intermediate four-hour period. A point of particular interest about the attraction of 1930 is the very low minimum temperature prevailing during the period of the greatest activity of Pink Bollworm moths, *viz.*, from 21st September to about the middle of October. The result was that much fewer moths came to the trap, and that the peak of attraction during this year was much lower than that of the other two years.

During 1931 the maximum attraction of the four-hourly periods also agrees closely with the optimum temperature prevailing in the various four-hour periods, but of a special interest is the high attraction of moths on a very few nights only. The low attraction during the early part of the season has already been explained, *viz.*, lateness of the crop, but the sudden fall in the attraction of moths from 21st October seems to be due to the combined influence of low temperature and, as will be shown later, lunar light.

Although maximum attraction agrees closely with the optimum temperature range, certain exceptions are noticed from Table III. On 2nd October 1929, for example, out of 140 moths collected only 59, or 40 per cent. were captured when the optimum temperature of 84-76° F. prevailed, while 74, or 55 per cent. of moths, were attracted in the last four-hour period when the temperature had gone down to 76°F. Again on 8th October 1929 of the 14 moths captured that night only 4, or 29 per cent., were collected when the temperature ranged between 91-71°F. while 9, or 64 per cent. were attracted when the temperature had gone much below the optimum zone.

Similarly, in 1930 on 23rd, 26th and 29th September and 25th October, the maximum number of moths was attracted during the intermediate four-hour period, while the optimum temperature had ranged during the first four-hour period.

In 1931 again the optimum temperature, on 23rd, and 30th September, ranged during the intermediate four-hour period when only 27 and 17 per cent. moths respectively were attracted, while in the last four-hour period 71 and 50 per cent. moths respectively were attracted when the temperature was as favourable if not less so.

The exceptions referred to so far relate to the larger attraction of moths after the time of optimum temperature range. On certain nights, on the other hand, it was found that although temperature within or very near the optimum zone ranged during the last four hours of the night, the number of moths captured then was very small. Thus on 2nd, 6th and 15th September, 1930 only 0, 11, and 15 per cent. moths were trapped during the last four-hour period when the temperature ranged from 78° to 75°F., 79° to 77°F. and 79° to 78°F. respectively. Similarly on 8th and 12th October 1931, only 6 and 17 per cent. moths were attracted during the last four-hour period when the temperature was within the optimum range.

Interpretation of these results presents some difficulties and the reasons are obvious. Phototropic response of insects is influenced by a number of environmental factors besides temperature, *e.g.*, movement of air and its strength, atmospheric humidity, perhaps pressure, moon, etc., and evidently variations must take place.

Another point that will be noticed from Table III is that on nights when four-hourly collections were made, moths, with few exceptions, were attracted throughout the night. This is due to the fact that temperature at Rohtak during the main period of the activity of Pink Bollworm moths does not go much above or below the optimum range, 76°F. to 87°F., and therefore the phototropic activity of these moths is not completely stopped. On a few nights at about the end of October and beginning of November, however, temperature during the last four-hour period went much below the suitable range, and consequently no attraction took place then.

At Cairo [Williams, 1924] the average minimum temperature goes down to below 68°F. in the month of September and October, and therefore, on the basis of our results, the favourable temperature for attraction there would be just after dusk.

VI. Influence of cotton picking on the attraction of *P. gossypiella*.

Cotton is picked at Rohtak from about the middle of September to the first week of November. During all the three years, 1929-1931, it was found that the number of moths trapped at light on the nights following the picking days was usually higher than those trapped either on the nights previous or subsequent. A reference to Table I will show that the largest number of moths trapped on any one night was 429 in 1929, 119 in 1930, and 402 in 1931, and all these nights followed the picking days.

In Table V the daily figures of attraction have been summarized in 7- or 8-day periods, and the average number of moths collected during the picking days is compared with that of the non-picking days in the same period. It is found that when the results of all the three years are considered together the mean number of moths collected per day during picking days is significantly higher than the mean for non-

picking days. The mean difference is 23·7 with a S. E. per cent. $\pm 4{\cdot}42$; Fisher's 't' works out to be 5·35 and the one per cent. value of 't' for $n=16$ is only 2·92.

TABLE V.

Collection of P. gossypiella *moths on picking and non-picking days.*

Periods		Number of		Number of moths captured on		Average number of moths captured per day	
		Picking days	Non-picking days	Picking days	Non-picking days	Picking days	Non-picking days
1929.							
September	8-15	4	4	289	121	72	30
	16-23	3	5	227	254	76	51
	24-30	3	4	590	652	197	163
October	1-7	3	4	315	370	105	93
	8-15	4	4	212	148	53	37
	16-23	4	4	68	58	17	15
	24-31	3	5	63	102	21	20
1930.							
September	8-15	2	6	111	37	56	6
	16-23	2	6	200	325	100	54
	24-30	3	4	214	160	71	40
October	1-7	2	5	49	88	25	18
	8-15	4	4	143	100	36	25
	16-23	2	6	66	130	33	22
	24-31	2	6	65	123	33	21
1931.							
October	1-7	2	5	232	364	116	73
	8-15	4	4	1,360	517	340	129
	16-23	2	6	161	156	81	26
	24-31	2	6	11	14	6	2

VII. EFFECT OF MOON ON THE ATTRACTION OF *P. GOSSYPIELLA*.

It is difficult to separate the influence of moon on attraction from that of temperature, picking and other environmental factors, since all of them act at one and the same time. There is, however, some evidence which goes to prove that on dark nights greater numbers of moths were attracted than on bright nights. Thus the periods of the highest attraction of all the three years (Table I), *viz.*, 23rd September to 7th October 1929, 15th September to 29th September 1930 and 5th October to 20th October 1931, all followed dark nights, but it is not possible

to say how much of this attraction was due to dark nights and how much to other factors. Similarly on bright nights in the periods from 12th October to 20th October 1929, 1st to 8th October 1930, and 21st to 30th October 1931, there was sudden fall in the attraction, but it is again not possible to ascribe this drop to moon only since the temperature, especially the minimum, fell considerably in this period.

VIII. Proportion of the sexes.

Willcocks [1916] found that out of the 5,856 moths attracted at an ordinary lamp, 42 per cent. were females and 58 per cent. were males. We determined the sexes of all the moths that were captured in 1930 and 1931, and out of the 2,359 moths caught in 1930, 780, or 33·1 per cent., were females, and in 1931, out of 3,084 moths caught, 1,285, or 41·4 per cent., were females, the rest were males. The proportion of males is, therefore, higher than that of females.

Of the females that came to light the majority were gravid; of the 780 females caught in 1930, 753 were dissected and 726, or 95·5 per cent., were gravid containing a very large number of both ripe and unripe eggs.

The proportion in which the sexes were attracted to light during various hours of the night was ascertained from the four-hourly collections made on certain nights. The majority of the females were caught between 18 and 22 hours, but the majority of the males came to the light after 22 hours, thus showing a slight sexual difference in their behaviours to light. Results are given in Table VI.

Table VI.

Proportion in which the sexes were attracted to light during the different hours of night.

Year	No. of nights under observation	Female moths collected						Male moths collected					
		18-22 hrs.		22-2 hrs.		2-6 hrs.		18-22 hrs.		22-2 hrs.		2-6 hrs.	
		No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
1930 . .	17	136	62·7	68	31·3	13	6·0	39	10·7	267	72·9	60	16·4
1931 . .	12	248	47·8	206	39·7	64	12·5	73	13·5	356	65·7	113	20·8

IX. Effect of light trap on the attack of *P. gossypiella*.

The possibility of utilizing light traps for the control of Pink Bollworm has been tested in several countries. Willcocks [1916] captured a very large number of moths with an ordinary lamp, but he came to the conclusion that the practical difficulty and expense of working a trap during July, August, September and perhaps also in October would be very great and sufficient to discourage most of the cotton growers from using them. Gough [1919] observed that the traps were not reliable measures of control.

Besides putting up the trap throughout the bolling season of cotton, we carried out a weekly examination of 250 green bolls from the field where the trap was put up, and for the sake of comparison a similar examination was carried out from another field about a mile away from the former. Both these fields were under similar conditions regarding date of sowing, watering and other agricultural operations. Results of this examination are given in Table VII.

It will be seen that green bolls for examination in 1929 were available from the first week of August, in 1930 they were available from the second week of August and in 1931 from the third week of August, that is to say, the crop of 1929 was the earliest and that of 1931 the latest. Further, the attack in 1929 was the highest, particularly in the plot where the light trap was kept. In 1931, on the other hand, the attack was the lowest of all the three years, especially in the beginning of the season, and reached appreciable proportions only after the first week of October.

Another point that will be noticed from Table VII is that the attack increased with the advance of season, but while the field away from the trap showed a regular rise of attack, in the field where the trap was set there was a very sudden rise in October, much higher than in the former plot. The light trap, therefore, instead of minimising the attack in the field where it was put up, only helped to concentrate the moths from the adjoining fields and thus increased the intensity of attack in that area. Further, the number of moths captured was not sufficiently high and the use of light trap cannot be advocated. Lastly, the difficulty of running the trap for several months, as pointed out by Willcocks [1916], is another great disadvantage. Light trap as a measure of Pink Bollworm control cannot, therefore, be recommended for the Punjab.

TABLE VII.

Comparative attack of Pink Bollworm of green bolls in plot with light trap and plot away from light trap.

Period	No. of *P. gossypiella* moths trapped			Percentage attack of green bolls in the plot with light trap			Percentage attack of green bolls in the plot away from light trap		
	1929	1930	1931	1929	1930	1931	1929	1930	1931
August—									
1—7	16	64	2	0	..	..	0	..	..
8—15	53	19	5	0	..	..	4·0	2·2	..
16—23	90	66	2	2·0	5·0	..	3·5	4·0	0
24—31	34	220	0	13·0	9·6	0·5	6·0	9·2	0·4
September—									
1—7	164	139	0	9·2	12·0	0·8	10·8	9·8	0·8
8—15	410	148	46	16·8	6·8	1·2	20·4	10·4	1·8
16—23	481	525	167	10·7	12·4	1·2	22·4	24·4	2·0
24—30	1,242	374	47	24·0	15·2	4·4	24·4	33·6	4·2
October—									
1—7	685	137	596	45·2	29·2	6·4	33·6	42·0	4·4
8—15	360	243	1,877	53·6	46·0	24·8	52·8	46·4	17·2
16—23	126	196	317	83·2	69·2	28·0	57·6	49·6	32·6
24—31	165	188	25	80·8	70·0	43·2	44·6	69·6	41·2
November—									
1—7	46	7	0	88·0	..	..	84·0	..	..
8—15	0	..	..	74·0	..	..	..	..	..
16—23	..	..	..	..	..	..	..	..	..
24—30	..	..	..	..	..	..	..	..	..

X. SUMMARY.

1. *P. gossypiella* moths exhibit positive phototropism in the Punjab. They may be trapped from about the middle of July to the first week of November, but the largest number collected during three years was from the middle of September to the middle of October.

2. Four-hourly collections made on certain nights showed that the moths were attracted throughout the night and that the period of the night when the greatest number of moths was attracted varied with the season.

3. It has been shown that the phototropic response depends largely on temperature and that the most favourable zone for attraction ranges from 76°F. to 87°F.

4. There was larger attraction on nights following picking days, than on the nights following non-picking days.

5. Moon appears to affect the phototropic activities of these moths.

6. Moths of both sexes were collected at the trap, the number of females was slightly less than that of the males. Dissection of the females showed that almost all of them were gravid.

7. Examination of green bolls showed that the light trap, instead of reducing the intensity of attack in the field where it was put, only served to increase it. It cannot, therefore, be recommended for the control of Pink Bollworm in the Punjab.

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CHROMOSOME NUMBERS IN THE GENUS *SACCHARUM* AND ITS HYBRIDS.

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(With Plates XVIII-XX)

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I. INTRODUCTION.

Work on the cytology of the *Saccharums* has been comparatively recent and mostly within the last two decades. Franck [1911], Kuwada [1915], Bremer [1923 *seq.*] and Artschwager, Brandes and Starrett [1929] are among those who have made material contributions to the subject. The contributions from Bremer have been of an outstanding nature, continuous for over a decade from 1923 and has broken considerable new ground on the subject.

With sugarcane the purely academic aspect has been somewhat neglected, chiefly owing to the fact that economic results through breeding have often been obtained by methods which are comparatively empirical. The mode of inheritance in this crop is as yet little understood [Venkatraman, 1927] and chance plays a great part in the breeding and selection of improved strains. It has even been doubted in certain quarters if it would ever become fruitful to try and synthesize economic types in this crop on Mendelian lines. On general considerations, however, it would appear likely that, ultimately, the cane might fall in line with other crops in the matter of inheritance.

The cytology of the sugarcane, though it may not be of immediate benefit as a guide to its breeding, is bound to play an increasingly useful part in the future. The Imperial Sugarcane Breeding Station at Coimbatore, which was started in the October of 1912 devoted practically the whole of its attention to the economic

aspect of breeding; and the results achieved would appear to have amply justified this position. But during the course of breeding all these years a considerable amount of very valuable and interesting material has accumulated, the latest being the intergeneric crosses between sugarcane and *Sorghum*.

In this paper the chromosome cytology of a portion of the accumulated material is briefly given and it is hoped to follow it up with similar contributions in the future. The main object of this paper is to present the results as obtained. No attempt is made to advance any theory, as it is felt that this is best done and, it would be time enough, when more material has been studied in a similar manner.

II. Material and methods.

In these studies the material was collected from a crop growing under wet land conditions. Immature arrows between the 'boenting'* and the 'flag stage' were collected in the mornings between 9 and 11 a.m. and on days with fairly good sunshine. After severance from the shoot, the arrow material was immediately fixed in Allen's modification of Bouin's fixative composed as below:—

Picric acid: saturated solution in distilled water	75 c. c.
Formalin	25 c. c.
Acetic acid	5 c. c.
Chromic acid	1·5 grms.
Urea	2 grms.

Merck's reagents were used for preparing the fixative. A weak solution of Flemming's fluid was also tried, but the one adopted was found cheaper and gave equally satisfactory results. Whenever it was found not practicable to fix the material in the field itself, the arrowing shoot with a good bit of the stem, was brought to the laboratory with the bottom end inserted in water immediately after cutting in the field. The fixative with the material immersed in it was put under the air pump to ensure quick and uniform penetration.

The material was left in the fixative for twenty-four hours and then washed in running water for a similar period. For selecting from the mass material at the right stage, it was stained with Belling's [1926] acetocarmine and examined under the microscope, when it was often possible to roughly count even the number of chromosomes. The smear method was also tried using Hæmatoxylin in place of Brazilin and it occasionally gave good results.

Microtome sections were cut between 8 and 10 μ and after taking to absolute alcohol, etc., in the usual manner, the slides were placed in saturated solution of

* 'Boenting' is the stage when a marked elongation of the throat of the shoot is noticed.

lithium carbonate in 70 per cent. alcohol for two to twelve hours to remove the yellow stain of the picric acid. Slides were stained in Haidenhain's Iron-Alum Hæmatoxylin and the chromosomes counted in the pollen mother cells from the polar view of the metaphase equatorial plate. Drawings were made with Zeiss Abbe apparatus on a Bernhard Drawing Table to a magnification of three thousand. In the published plates the drawings are magnified to one thousand five hundred, the originals being reduced to half size.

III. Chromosome numbers in wild saccharums (Plate XVIII).

A certain number of wild *Saccharums* has been accumulated at the station including a fair collection of the types occurring in India and a set of rather interesting specimens kindly presented by the Hawaiian Delegation to the International Society Conference in Java in 1929.

Bremer [1924] found thirty bivalents in *Saccharum Munja* and one form collected from the Punjab showed this number (Fig. 2). Another form of *Saccharum Munja* collected from Karnal by Mr. R. Thomas showed, however, only ten bivalents (Fig. 1). The types collected under *Saccharum spontaneum* showed variation in the number of chromosomes (Figs. 3-8). Venkatraman [1929, 1930, 1931, 1932 and 1933] found these forms to differ from one another not only in their morphological and root characters, but interesting differences were noticed in the resultant hybrids even when crossed with the same *Saccharum officinarum*. The number of chromosomes was found to vary from twenty-seven in the Dehra Dun form to as many as sixty-four in the thick-stemmed form from Sumatra (Table I).

Table I.

Chromosome numbers in different forms of Sachh. spontaneum.

Type of *Saccharum spontaneum*	Number of bivalents
Dehra Dun form	27
Rella gaddi (Godavary, Madras)	32
Coimbatore form	32
Dacca form	39
Thin-stemmed form, Sumatra	54
Thick-stemmed form, Sumatra	64

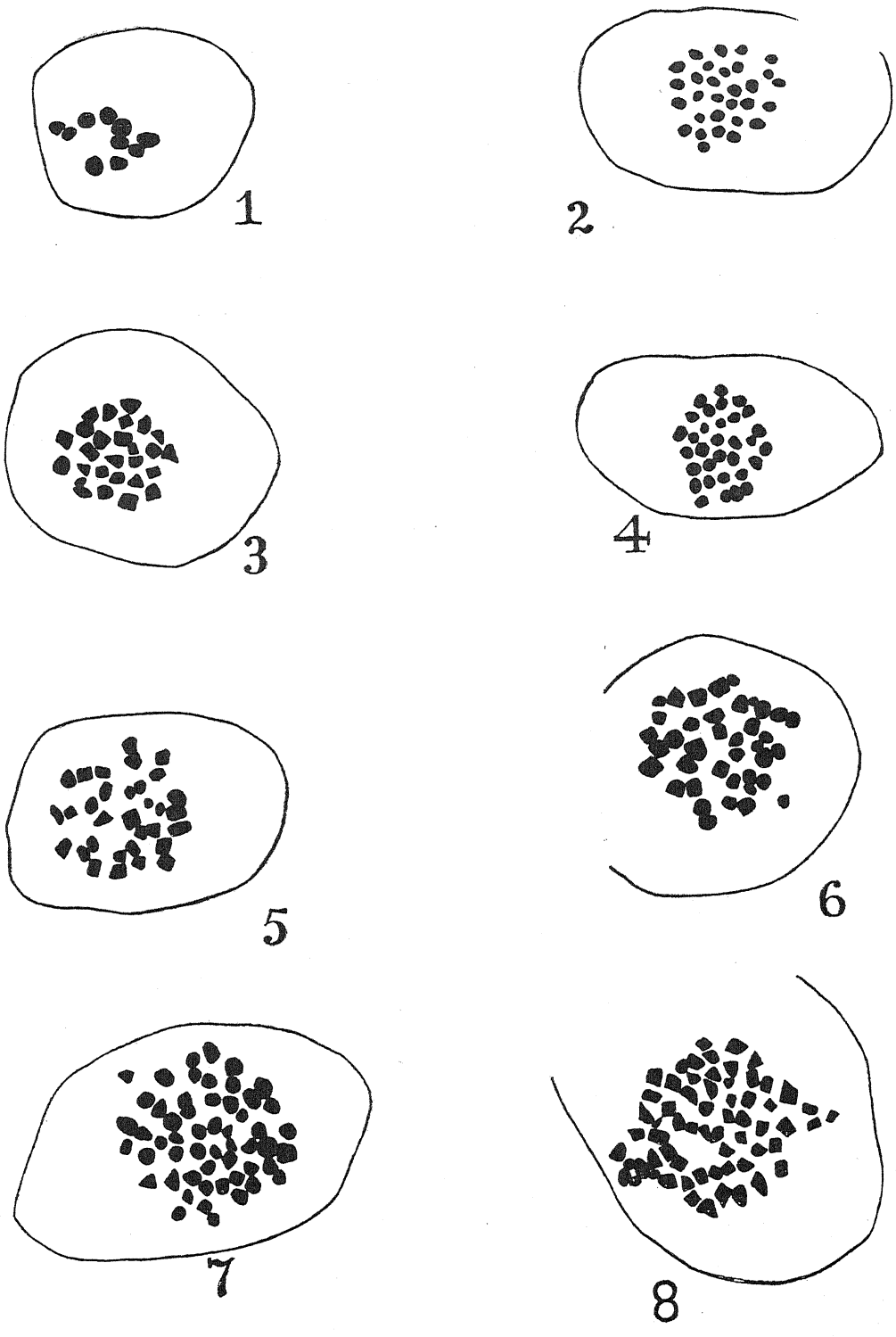
The thick-stemmed form from Sumatra is definitely the largest form of the series and happens to possess the largest number of chromosomes as well.

IV. Chromosome numbers in saccharum hybrids (Plate XIX).

(*a*) *Vellai* × *Saccharum Narenga* (*Figs. 1-4*).—Hybrids between *Vellai*, a noble cane with forty bivalents [Dutt, 1933] and *Saccharum Narenga* with fifteen biva-

WILD SACCHARUMS

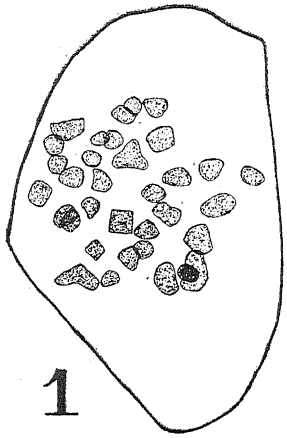
(Polar view, metaphase, haploid.)

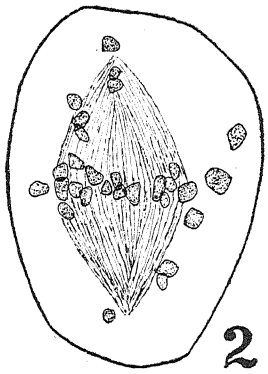


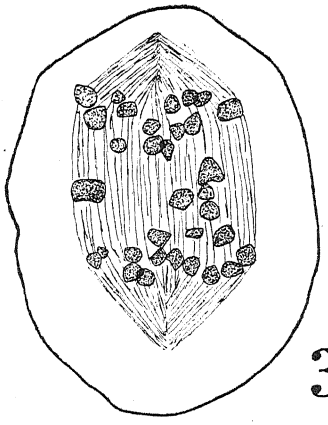
1 & 2.—Two different forms of *Saccharum munja*.
3.—*Sacch. spontaneum* (Dehra Dun). 4.—*Sacch. spontaneum* (Godavery, Madras).
5.—*Sacch. spontaneum* (Coimbatore, occurring on canal bunds).
6.—*Sacch. spontaneum* (Dacca, Bengal). 7 & 8.—Thin-stemmed and thick-stemmed forms of *Sacch. spontaneum* (?) from Sumatra.

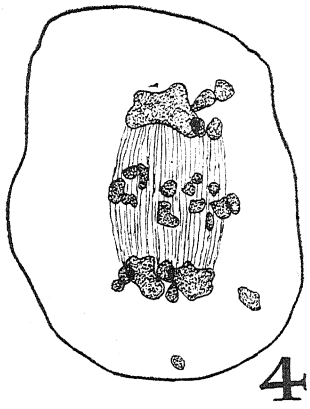
PLATE XIX.

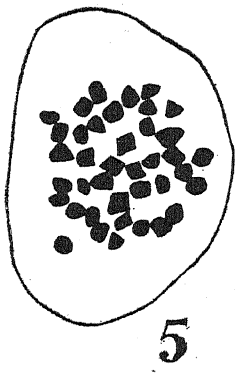
SACCHARUM HYBRIDS

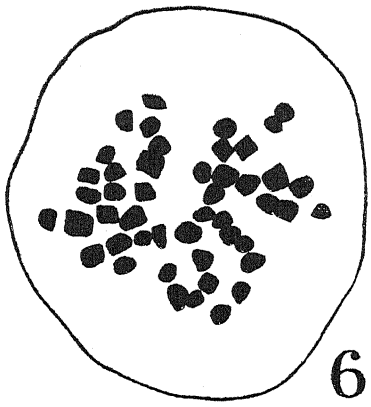


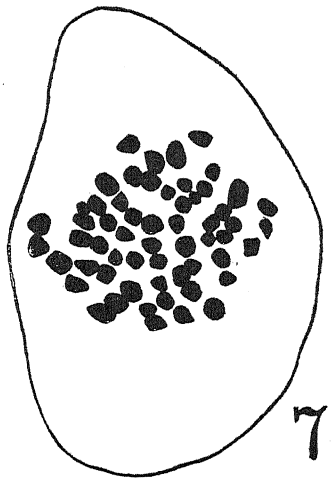












1, 2, 3 & 4.—Vellai × *Saccharum narenga*.
1.—Polar view, metaphase, haploid. 2.—Metaphase side view showing extruded chromatin.
3.—Anaphase side view showing certain of the chromosomes lagging behind.
4.—Telophase showing both extruded chromatin and chromosomes lagging.
5.—Saretha × *Sacch. spontaneum*. Polar view, metaphase, haploid.
6.—Shakarchynia × *Sacch. spontaneum*. Polar view, metaphase, haploid.
7.—P. O. J. 2725 × *Sorghum Durra* Stapf. Polar view, metaphase, haploid.

lents [Bremer, 1924] are of some interest. These hybrids have been propagated from cuttings now for nearly two decades. While in the earlier years they exhibited pratically complete sterility in the male, they would now appear to show a slight tendency to pollen formation. As would be seen from the illustrations the reduction division is irregular with quite a large number of univalents. One often sees in such material chromosomes lagging behind in the telophase and anaphase stages. Univalents are rather large and remind one of the descriptions and illustrations of *Hitam Rokan* by Bremer [1924]. It is a tribute to the genius of Dr. Bremer that he should have suspected *Hitam Rokan* to be a hybrid between a noble cane and *Saccharum Narenga* from an examination of the chromosomes. The chromosomes figured (1) are of a cross deliberately made between the noble cane *Vellai* and *Saccharum Narenga* and are 34 in number (including univalents and bivalents). In this case there is no doubling on the *Vellai* side as in the cross between *Vellai* and the Coimbatore form of *Sacch. spontaneum* [Dutt, 1933].

(*b*) *Saretha* × *Sacch. spontaneum* (*Fig. 5*).—The chromosome number of *Saretha* has been worked out by Bremer [1932] to be 92 somatic and of *Sacch. spontaneum* by Dutt to be 32 haploid. The hybrid between *Saretha* and *Sacch. spontaneum* shows 39 bivalents (Fig. 5) indicating that there is no doubling of chromosomes in either of the parents, *i.e.*, $\frac{\frac{92}{2}+32}{2}$ or 39 haploid.

(*c*) *Shakarchynia* × *Sacch. spontaneum* (*Fig. 6*).—*Shakarchynia* is an unclassified indigenous cane of Bihar whose chromosome number has not yet been determined. A hybrid between this cane and the Coimbatore form of *Sacch. spontaneum* showed 47 bivalents (Fig. 6). The Coimbatore form of *Sacch. spontaneum* is known to possess 32 bivalents. If the chromosome behaviour in this cross should be similar to that in *Saretha* × *Sacch. spontaneum* the haploid number in *Shakarchynia* should be twice 47 *minus* 32, or 62. If so, this cane might have affinity with the *Nargori* group of Indian canes in which Bremer [1932] has recorded 124 as the somatic number. It is rather unfortunate that the cane *Shakarchynia* is not now available in the locality from which it was originally collected, as the same has been overrun by the higher yielding Coimbatore canes. An attempt is, however, being made to collect the cane and study its pollen mother cells.

(*d*) *Sugarcane* × *Sorghum hybrids* (*Fig. 7*).—The only intergeneric cross between sugarcane and *Sorghum* studied so far is that between P. O. J. 2725 and *Sorghum Durra* Stapf. These hybrids were found to exhibit great irregularity in the behaviour of the chromosomes and a fairly normal type is illustrated (Fig. 7). Bremer [1928] recorded 106 somatic chromosomes in P. O. J. 2725 and 10 (haploid) has been recorded for *Sorghum* [Longley, 1932]. The hybrid examined showed 58

bivalents postulating a doubling of the chromosomes on the sugarcane side, thus, $\frac{2\times53+10}{2}=58$.

V. Chromosome numbers in Co. 213 and its bud sports (Plate XX).

Thomas [1932] found a series of bud sports in this cane leading back to the original Co. 213. It is interesting and gives food for thought that these vegetative sports should show differences in chromosome numbers as well. The original Co. 213 (Fig. 1) shows 59 bivalents. A striped sport was obtained from Co. 213 which on the whole is a trifle less vigorous than the original Co. 213. This striped sport shows 62 bivalents. On cultivation this striped sport threw three distinct bud sports :—

1. A thick-stemmed form with 58 bivalents very similar to the original Co. 213 (Fig. 3) ;
2. A distinctly thinner form with 59 bivalents otherwise similar to the original Co. 213 (Fig. 4), and
3. A thin unicoloured wax-yellow form with 46 bivalents (Fig. 5).

Of these hybrids, the thin yellow form is the poorest and is gradually losing its vigour. It happens to possess the smallest number of chromosomes as well.

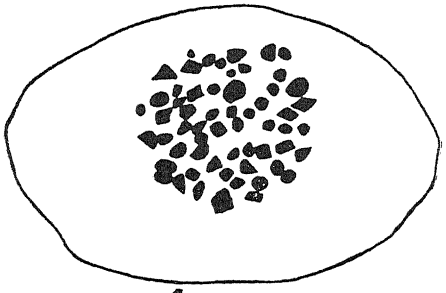
My thanks are due to Rao Bahadur T. S. Venkatraman, B.A., I.A.S., and Mr. R. Thomas for help and advice. A good deal of the material referred to in the paper was under the direct charge of Mr. R. Thomas who had been carefully planting it out from year to year for well over a decade.

VI. REFERENCES.

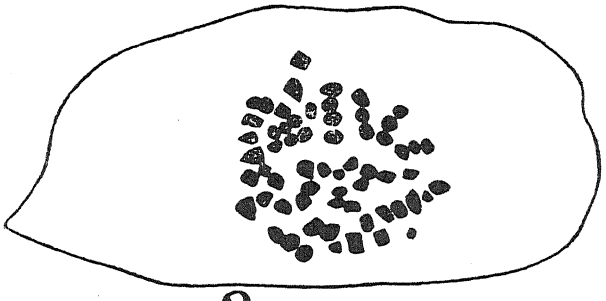
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CO. 213 AND ITS 'BUD' SPORTS

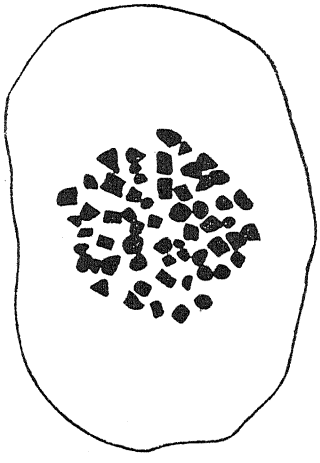
(Polar view, metaphase haploid.)



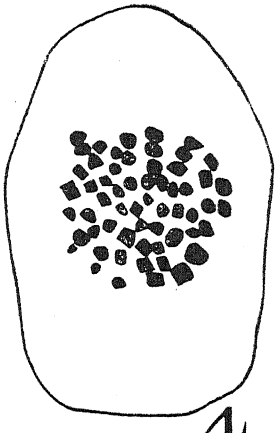
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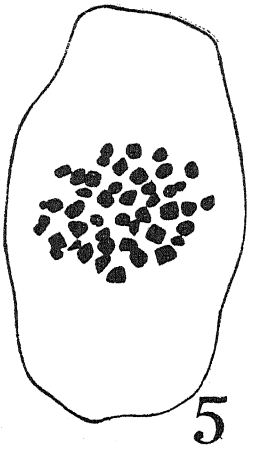
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3

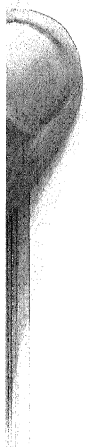


4



5

1 = Co. 213; 2 = 'Striped sport' from Co. 213;
4, & 5 = 'Thick Red' 'Thin Red' & 'Thin yellow' Sport from 2.



VARIATIONS IN THE CHARACTERS OF COTTON FIBRES WITH THE PROGRESS OF THE SEASON.

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(With three text-figures)

I. Introduction.

Many attempts have been made to investigate the variations in the fibre properties of cotton picked during different parts of the season. Generally, in those cases, the whole crop has been picked in three different lots and the lint therefrom tested for average fibre length by the halo-method. In some cases the average ribbon width and the average breaking strength of a fibre have also been determined. The data thus obtained have always shown an increase in the average length and a decrease in breaking stress towards the end of the season.

In the present paper it is intended to trace qualitatively the effects of the climatic conditions on the cotton crop in so far as they induce variations in fibre characters with progressive pickings.

The observations in the case of these experiments were made during two successive seasons. The experiments during the first season (1930-31) were intended to serve as a preliminary test which, however, yielded some encouraging results. The detailed experiments were, therefore, carried out during the next season, *viz.*, 1931-32.

II. Material and sampling.

The material for these studies consisted of three Punjab-American cottons, *viz.*, Early Strain, 4 F and 289 F, grown in the "Physiology Plot" of the Cotton Research Farm, Lyallpur, during the seasons 1930-31 and 1931-32.

In the "Physiology Plot" cotton is always grown after Berseem, the last cutting of which is buried in the field in February. The Plot is left fallow for one season after cotton, so that the rotation is Berseem——→cotton——→fallow. As regards irrigation to the cotton crop, water is given when the soil temperature at 30 cm. depth goes above 35°C. In 1930-31, 4 F and 289 F were sown on the 2nd May 1930. In 1931-32, all the three varieties were sown on the 8th May 1931.

During the season 1930-31, 20 plants were labelled at random from each variety. Care was taken to exclude the border plants to avoid boundary effect. The open bolls from those plants were collected every two weeks. There were 5 pickings for each of Early Strain and 4 F, while there were 6 pickings for 289 F. During 1931-32, 20 plants of the same three varieties were similarly labelled and the open bolls were again collected every two weeks. There were 8 pickings for Early Strain, 7 for 4 F, and 6 for 289 F. Of the seven pickings in the case of 4 F, the 1st picking gave only one badly opened boll. Consequently the actual number of pickings utilized for the present purpose was 6 for 4 F also.

For each picking the badly opened bolls were rejected and the sample for experimental work was drawn from the fluffy well-opened bolls alone. The number of such bolls available for each pick is given in Table I.

TABLE I.

Number of well-opened bolls available for tests.

Date of picking	1930-31			Date of picking	1931-32		
	Early Strain	4 F	289 F		Early Strain	4 F	289 F
19th September 1930	7	..	..	24th September 1931	1	..	..
4th October ,,	88	22	2	8th October ,,	1	..	..
20th ,, ,,	64	27	4	24th ,, ,,	22	12	3
3rd November ,,	34	72	56	5th November ,,	78	32	22
18th ,, ,,	9	104	164	19th ,, ,,	34	27	4
3rd December ,,	..	29	27	3rd December ,,	40	34	55
18th ,, ,,	..	..	6	17th ,, ,,	6	3	29
				2nd January 1932	9	1	22

The method of sampling was simple but rigid. A random sample was obtained from the seed cotton of the well opened bolls by the method of subdivision*. This

*The seed cotton from the well opened bolls was divided into two parts. Each of these two parts was again divided into two, and the process of subdivision was continued till the whole lot was split up into 16 parts. Then a few seeds were picked up at random from each of these parts making up the experimental sample. In those cases in which the number of bolls was very small, all the available seed cotton was utilized to make up the sample.

sample was weighed and then ginned on a hand driven gin. The lint and the seed were again weighed separately and the seeds were then counted. From this lint two slivers were independently prepared, with the help of the Balls Sorter Draw box, and tested for average fibre length and fibre length distribution by Balls Sorter. A hand made sliver carefully prepared from the same lint was utilized for the determination of fibre weight per unit length (by "cutting" method) and fibre maturity ("caustic soda" method). In addition to these, in the case of Early Strain a thin hand sliver was prepared by repeated drawing, doubling and rejecting one half of the doubled sliver, and the fibres in one cross-section of this sliver were all tested for torsional rigidity (by Searle's method). Another thin sliver of Early Strain was also similarly prepared for obtaining the single fibre breaking strength (by O'neill Tester).

It may be stated here that the reason for preparing separate hand slivers for testing either fibre rigidity or fibre strength was that these two properties might possibly be altered by the passage of a sliver through the draw-box.

III. Experimental and theoretical methods.

(*i*) Seed weight and lint weight were found by direct weighment on a sensitive physical balance.

(*ii*) Average fibre length was determined with Balls' Sledge Sorter. The result for each picking is the mean from two independent slivers.

(*iii*) Mean fibre weight per cm. was obtained by the "cutting" method. The total number of fibres tested for each sample was 1,200. These fibres were weighed in 4 lots of 300 each on a quartz micro-balance whose average sensitivity was 0·050 mgrm. per division. The 300 fibres of each lot were drawn out of 3 tufts of cut fibres at the rate of 100 fibres from each. The three tufts were drawn successively from a well sampled sliver, each of them constituting all the fibres in a cross-section.

(*iv*) Fully ripe and immature fibres were counted by the technique described by Clegg [1930]. 300 to 500 fibres were examined for a sample corresponding to each date of picking.

(*v*) Fibre strength determinations were carried out with the O'neill Tester. All the fibres, about 100 on the average, in a cross-section of a thin sliver, were tested. As there was no laboratory hygrometer available at the time of these experiments, Calcium chloride solution for 40 per cent. relative humidity was constantly used in the tube.

(*vi*) Torsional rigidity was found out by Searle's method ; the values obtained correspond to the expression, $\frac{8\pi^3 IL}{T^2}$, where I is the moment of inertia of the vibrating aluminium rods, L is the length of the fibre under test, and T is the period of torsional oscillations. In these determinations also, all the fibres—about 60 on the average—from one cross-section of a thin hand-made sliver were tested. The relative humidity inside the box containing the suspended fibres, was controlled at 70 per cent. by calcium chloride solution of density $=50{\cdot}4°$ *Tw*. The fibres were kept in this atmosphere for about 24 hours before measuring $\frac{L}{T^2}$.

(*vii*) In order to calculate the number of hairs of different lengths per seed, *i.e.*, the number of hairs per seed having lengths in the proportion indicated by the Balls' sorter distribution, a formula derived by the author in the following manner was utilized.

Let w_1, w_2,.....be the weight percentages of the Balls sorter sections of mean lengths l_1, l_2,..... Let f be the fibre weight per unit length (regarded constant for fibres of all lengths as well as along the length of any fibre). Then the number of fibres in the Balls sorter sections or n_1, n_2,.....are given by

$$n_1=\frac{w_1}{fl_1},\ n_2=\frac{w_2}{fl_2},\ \ldots\ldots$$

If $\Sigma n=n_1+n_2+\ldots\ldots$, then $\Sigma n=\frac{1}{f}\ \Sigma\ \frac{w}{l}$.

Also $\Sigma w=w_1+w_2+\ldots\ldots=100$.

Now, 100 units of weight of lint give Σn fibres. Therefore, actual lint weight, W, will give $\frac{\Sigma n}{100}\times W$ fibres. Let S be the number of seeds producing lint of weight W. Then the number of fibres of different lengths per seed is given by $\frac{\Sigma n}{100}\times\frac{W}{S}=\frac{1}{100}\cdot\frac{W}{S}\Sigma\frac{w}{l}$. If $L=$ lint weight per seed, $L=\frac{W}{S}$.

Hence the total number of fibres of different lengths per seed $=\frac{1}{100}\cdot\frac{L}{f}\Sigma\frac{w}{l}$

[*cf.* Burd, 1924].

(*viii*) Modal length was determined by calculating the median from the Balls sorter distributions, and using the formula :

Mode—Mean = 3 (Median—Mean).

(*ix*) Fibre length irregularity [Ahmad, 1932] was obtained from the Balls sorter distributions by finding out the percentages of all fibres that are shorter than three-fourths of the calculated modal length.

IV. Summary of earlier works.

(*a*) *Seed weight and ginning outturn.*

Although with regard to seed weight Balls [1912, 1] stated that " this weight is completely determined by the mother plant and not by the embryo ", he seems to have partly changed his view when he stated [Balls, 1915, 1] that " seed weight is a feature which to some extent is determined by the size of the seed, which is settled at the same time as lint length and should therefore fluctuate with it ". With regard to ginning outturn Balls [1930] pointed out the primary dependence of ginning outturn on the number of hairs on a seed. Kottur [1929] from his studies on Dharwar cottons could not find any relationship between average fibre length and ginning outturn. Barritt [1930] is of opinion that " the rise in the ginning outturn in late autumn is due to the effect of nutrition on the growth of the hair cells alone."

(*b*) *Average length.*

Balls [1912, 2], Afzal [1930] and Ware [1929] have respectively shown that lint length is a heritable character; consequently environmental conditions, such as climate, watering and cultural operations can produce variations in lint length within the limit fixed by heredity. That within this limit the first half of the maturation period determines the length to which cotton lint will attain, has been emphasised by Mayton *et al* [1931] as well as by Kelsick [1917, 1].

(*c*) *Fibre weight per unit length.*

Campbell [1929, 1] found that " fibre weight per unit length increased with the progress of maturity from fertilization until the opening of bolls..... Secondary thickening is responsible for two-thirds of the fibre weight of ripe hairs.. .....The short hairs are heavier in proportion to their length than the long ones."

(*d*) *Nutrition of seed hairs, fibre maturity and wall-thickening.*

Barritt [1929] states that " the growth of the hairs is dependent on the intercellular sap which escapes through the stomatal pores of the seed-coat ". Farr [1931] is on the contrary of opinion that " the nutrition of the hair in any other fashion than through its basal connection with the seed seems to be quite unlikely ". She states that " an examination of bolls in all stages of development fails to reveal any liquid or semi-liquid substance in the boll cavity ". But this categorical denial about the presence of a fluid cannot be regarded as wholly established and much more work is necessary in this direction. It may, however, be stated that young bolls on being opened artificially do, more often than

not, exhibit a slimy fluid in the boll cavity. Whether this fluid takes any active part in the nutrition of the seed hairs or not, the present writer is not aware.

It is stated elsewhere [*U. S. A. Dept. Agric. Bull. No.* **33**] that the seeds which, in a very immature boll, are attached to the inner angle of each loculus inside the boll cavity, ' retain this attachment until they have nearly reached their mature size and growth of the lint has begun on them '. At that point ' their attachments begin to be absorbed and by the increased growth of the lint the seeds are forced into the centre of the cavity '.

Balls [1928] states that ' during the first half of the maturation period fibres grow in length ; later on growth in length ceases, but the process of growth continues in the direction of thickening of the fibre walls,' the whole process behaving " as if a switch has been thrown over releasing a different set of growth machinery." This view has been supported by the recent work of Sakostchikoff and Karsheniovsky [1932]. It will not therefore be surprising if future biological research will establish that the fibres get their nutriment through " basal connection with the seed ", as Farr suggests, during the first half of the maturation period, while they get their nutriments in the manner suggested by Barritt during the other half.

Hawkins [1931, 1] states that " wide variations in fibre maturity occur in seeds of similar appearance ". Hawkins and Serviss [1930, 1] found " no appreciable thickening of the fibre walls until fibre elongation was almost completed ".

(*e*) *Breaking strength and torsional rigidity.*

Campbell [1929, 2] found that " increase in fibre weight per unit length is accompanied by increase in tensile strength" . Pickard [1927] states that " Rigidity is closely related to hair weight per cm. both on the average of different varieties and in variation within a small sample ". According to Balls [1915, 2] " the breaking strain of a fibre is very largely determined, if not entirely, by its weight or in other words, by the thickness of its cell-wall."

(*f*) *Picking and quality.*

Zaitsev [1925-27] states that apart from nutrition the " fundamental external factor that definitely disturbs the otherwise rhythmical cycle of the cotton plant " is weather or seasonal condition. According to him " the higher the temperature the more rapid is the development and the ripening of the boll ; the lower the temperature the longer the boll takes to reach its final stages ". The work done by Mell [1900] and Smith [1920] indicates that ' the cotton plant requires a favourable temperature and reacts strongly to falling tempera-

tures, while too much rainfall and cloudy weather is in general unfavourable.' It is interesting to study the works of Ballard and Simpson [1925] and of Gilbert [1926] in this connection.

Considering the average length of lint of bolls opening on a particular date and the four previous days Kelsick [1917, 2] concludes that bolls opening later give longer lint. He also found in Egypt that there is a rise in lint length a few days after the plants have been irrigated. Kearney and Harrison [1924] found " in 33 individual plant selections of the Yama variety of Egyptian cotton grown in Arizona, that fibre from the second picking averaged $\frac{1}{16}$ inch longer than fibre from the first picking". De Balsac et Miege [1930] by their studies particularly confirm " the superiority of the first crop over the following ones for the same variety, under the same cultivation and at the same place ". Ramanatha Iyer and Jagannatha Rao [1930] observed that " there are definite differences in the lint lengths of seeds produced in different pickings " but they found " no definite relation between lint length and date of pickings."

According to Hawkins [1931, 2] " the last picking usually contains the greatest amount of immature fibres. The percentage of immaturity increased as the season advanced irrespective of the frequency of irrigation ".

Venkataramanan [1930] working at Coimbatore found " a general fall in maturation period, seed weight, lint length and lint weight towards the later formed bolls ". He also found that " the decline varies in different periods of the same season and differently with the characters ". He showed further that the shortening of the maturation period consequent on a rise of temperature is generally accompanied by a deterioration in character " (lint weight, seed weight or lint length). He states that the " spinning tests also show that earlier picked cotton is better than the late pickings from the same field of a pure strain ".

Hawkins and Serviss [1930, 2] state that " prevailing temperature contributes to the rate of fibre development, but when the former is below an optimum it has a retarding effect on length and fibre thickening. The rate of fibre-wall thickening became less as temperature declined ".

V. Experimental results and their discussions.

Although the results for the season 1930-31 indicate some change in the physical properties of the fibres yet the change is not so marked as for the season 1931-32. In using the results of 1930-31 it is necessary to bear in mind the observations of Trought and Afzal [1931] who say in connection with their experi-

ments regarding the sowing date of the Punjab-American cottons at Lyallpur that "the results [1930-31] differ from those of the previous year. This is attributed to the particularly mild season."

Consequently in the present analysis the results of 1930-31 may not be considered beyond the point that they indicate some definite change in the qualities of the cottons with the progressive pickings.

With regard to Early Strain and 4 F, the same authors point out that "there are marked differences between 4 F and Early Strain both morphologically and physiologically."

Consequently 4 F must be studied as a distinct case which may be expected to vary from the others at least with regard to some characters.

The pickings on different dates have been numbered for the sake of convenience in the manner shown in Table II. The experimental results of all the three varieties appear in Table III.

TABLE II.

Pick No.	Dates of picking	
	1930-31	1931-32
1	19th September 1930 . . .	24th September 1931.
2	4th October 1930	8th October 1931.
3	20th October 1930	24th October 1931.
4	3rd November 1930	5th November 1931.
5	18th November 1930 . . .	19th November 1931.
6	3rd December 1930	3rd December 1931.
7	18th December 1930 . . .	17th December 1931.
8	——	2nd January 1932.

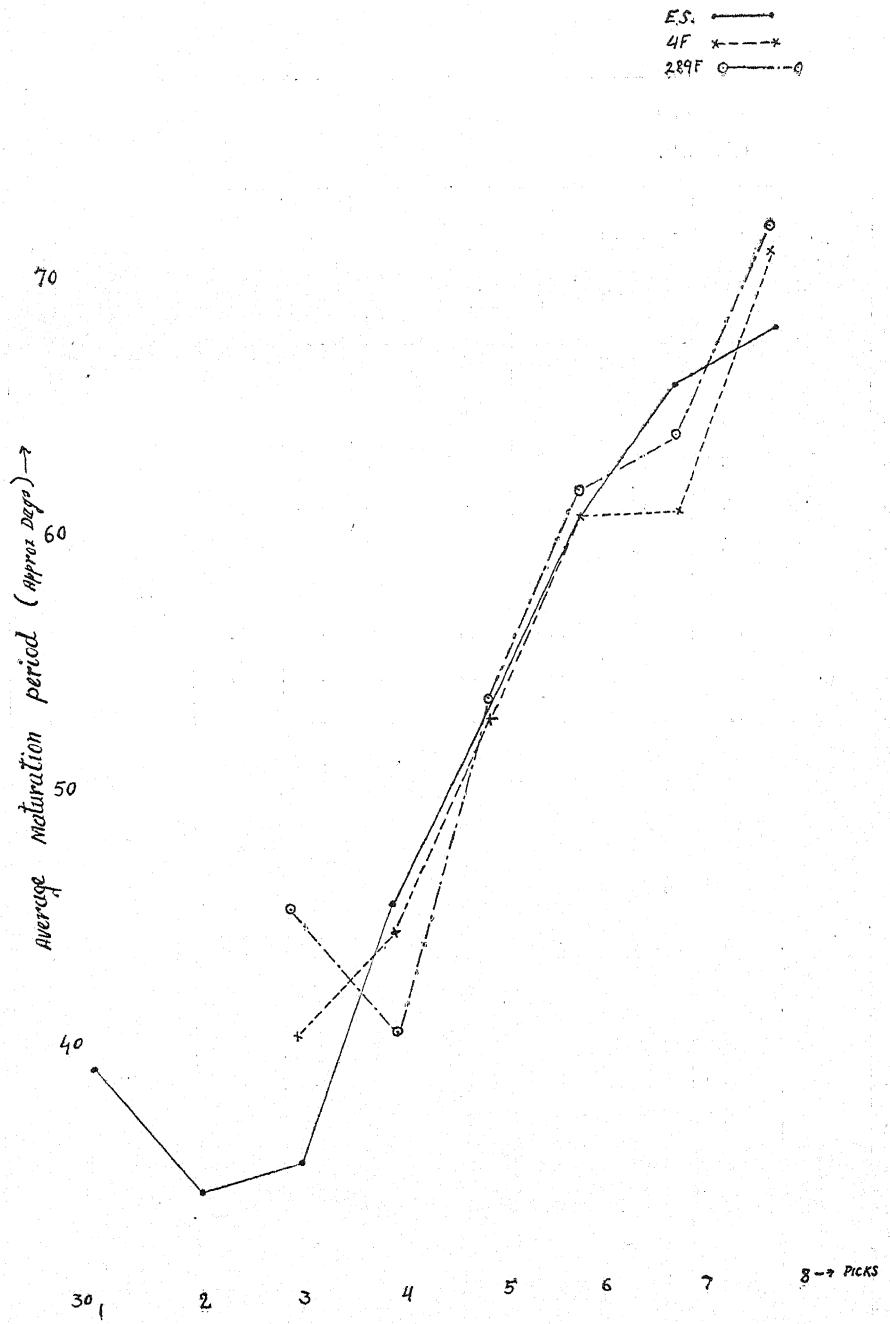

E.S.
4F
289F
70
60
50
40
30
Average maturation period (approx Degs)→
1
2
3
4
5
6
7
8→ PICKS

Fig. 1.

TABLE

Experimental values for

Pick No.	Seed index (grms.)	Lint index (grms.)	G. O. T. (percentage)	Average fibre length (cm.)	Fibre wt. per cm. (grms.) $\times 10^{-6}$	Fully ripe hairs (percentage)	Dead hairs (percentage)	Fibre rigidity (dynes cm^2.)	Fibre strength (grms.)	P.-A. 4F. (1930-31). Seed index (grms.)	P.-A. 4F. (1930-31). Lint index (grms.)
	Early Strain (1930-31).										
1 .	7·1	3·2	30·9	2·52	1·78	63	11	..	..	..	..
2 .	6·2	3·1	32·9	2·54	1·51	67	10	..	..	7·7	2·5
3 .	6·6	3·2	32·9	2·34	1·83	63	10	..	..	7·6	4·1
4 .	6·1	3·0	33·4	2·36	1·76	77	8	..	..	6·9	3·4
5 .	6·7	3·0	31·0	2·41	1·68	67	14	..	..	7·5	3·5
6 .	..	..	..	..	..	..	..	..	..	7·7	3·0
7 .	..	..	..	..	..	..	..	..	..	..	..
8 .	..	..	..	..	..	..	..	..	..	..	..
S. E. .	0·185	0·045	0·527	0·041	0·056	2·46	..	..	..	0·161	0·254
	Early Strain. (1931-32).									P.-A. 4F (1931-32).	
1 .	7·9	3·3	29·5	2·31	1·67	69	9	13·31	1·99	..	..
2 .	7·4	3·7	33·3	2·51	1·35	41	19	6·60	2·11	..	..
3 .	9·3	3·7	28·5	2·51	1·76	69	9	10·41	2·65	6·0	2·8
4 .	9·2	3·5	27·6	2·56	1·53	58	12	9·33	2·46	6·6	2·6
5 .	8·6	3·6	29·5	2·51	1·65	55	15	11·70	1·77	7·0	2·8
6 .	8·2	2·9	25·9	2·36	1·41	43	21	6·14	1·78	7·3	3·0
7 .	9·2	2·7	22·7	2·39	1·34	40	19	4·34	1·81	6·8	2·8
8 .	8·8	2·8	24·1	2·21	1·24	33	26	3·58	1·59	7·6	2·8
S. E. .	0·244	0·148	1·191	0·017	0·066	4·80	..	1·25	0·130	0·230	0·047

III.

all the three varieties.

					P.-A. 289F (1930-31).						
G. O. T. (percentage)	Average fibre length (cm.)	Fibre wt. per cm (grms.) X10^{-6}	Fully ripe hairs (percentage)	Dead fibres (percentage)	Seed index (grms.)	Lint index (grms.)	G. O. T. (per centage)	Average fibre length (cm.)	Fibre wt. per cm. (grms.) X10^{-6}	Fully ripe hairs (per centage)	Dead hairs (per centage)
..	..	..	..	..	..	..	..	..	..	..	..
24·8	2·06	2·20	70	17	7·6	3·4	30·9	2·62	1·67	89	2
34·9	2·06	2·17	74	15	7·4	3·6	32·7	2·54	1·56	70	9
32·8	2·06	2·13	65	20	8·2	3·5	30·0	2·57	1·62	84	6
31·8	2·11	2·24	64	21	8·3	3·3	28·5	2·59	1·62	82	5
28·1	2·18	1·77	65	20	8·3	2·9	26·0	2·46	1·56	70	11
..	..	..	..	..	9·3	2·7	22·3	2·49	1·73	62	10
..	..	..	..	..	..	..	..	..	..	..	..
1·798	0·024	0·085	1·86	..	0·277	0·149	1·531	0·025	0·027	4·18	..
					P.-A. 289F (1931-32).						
..	..	..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..	..	..
31·8	1·75	2·00	60	19	9·6	4·1	29·9	2·61	1·88	83	5
28·3	1·90	1·94	49	21	9·7	3·5	26·5	2·61	1·76	73	4
28·6	1·96	2·20	51	18	9·1	3·1	25·4	2·69	1·61	65	2
29·3	1·85	2·27	61	13	9·1	3·2	26·0	2·61	1·56	64	9
29·2	1·68	2·35	80	4	9·3	3·9	25·6	2·61	1·70	76	5
27·0	1·68	2·04	63	10	8·8	2·9	24·8	2·56	1·33	56	10
0·65	0·019	0·067	4·44	..	0·140	0·171	0·744	0·007	0·077	3·98	..

From the graphical representation in Fig. 1 representing the period of maturation of bolls opening between two consecutive pickings, it is observed that the maturation period of all the varieties after some fluctuations in the beginning, rose in a fairly steep curve as the season advanced, compatibly of course with the rapidly falling temperature during the bolling season at Lyallpur.

(*1*) *Seed index, lint index and ginning outturn.*—Referring to Figs. 2 and 3 it is found that the seed index for 1930-31, which was a particularly mild season, did not show much progressive change as the season advanced except in the case of 289 F. For this cotton (289 F) the seed index increased considerably with the advance of the season. For 1931-32 the seed index in the case of Early Strain, showed a tendency to rise with the progress of the season while it showed a considerable increase in the case of 4 F. The tendency in the case of 289 F was, however, towards a decrease with the advance of the season. Consequently nothing can be definitely said regarding the variation in the seed index with the progress of season. Not so however, in the case of lint index and ginning outturn. These characters generally showed a definite tendency during both the seasons to decrease towards the end of the season. From Table VI it can be seen that there is no significant correlation between seed index and either average fibre length or fibre maturity.

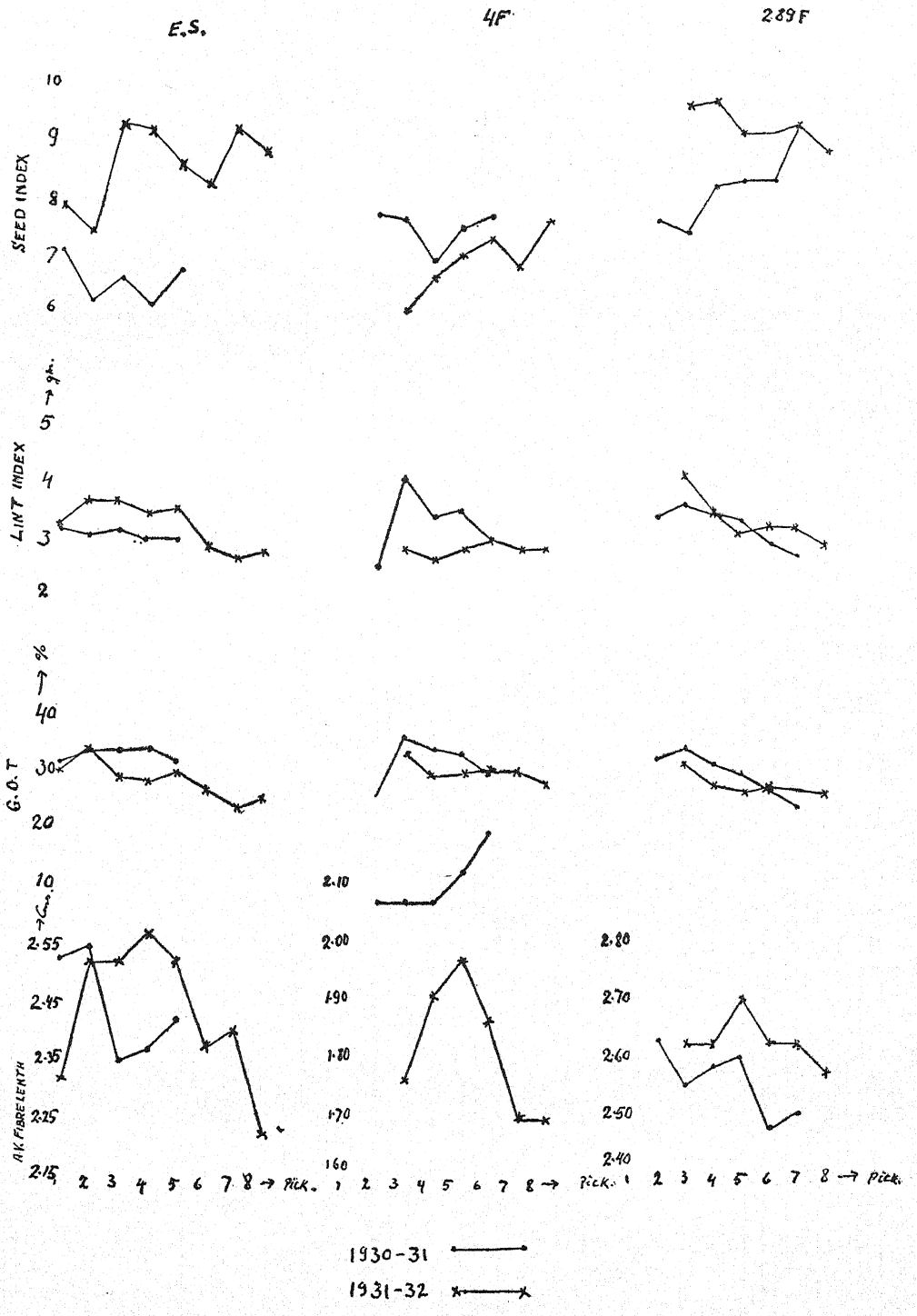

E.S.
4F
289F
SEED INDEX
LINT INDEX
→ gm.
G.O.T
→ %
AV. FIBRE LENGTH
→ Pick.
1930-31
1931-32

Fig. 2.

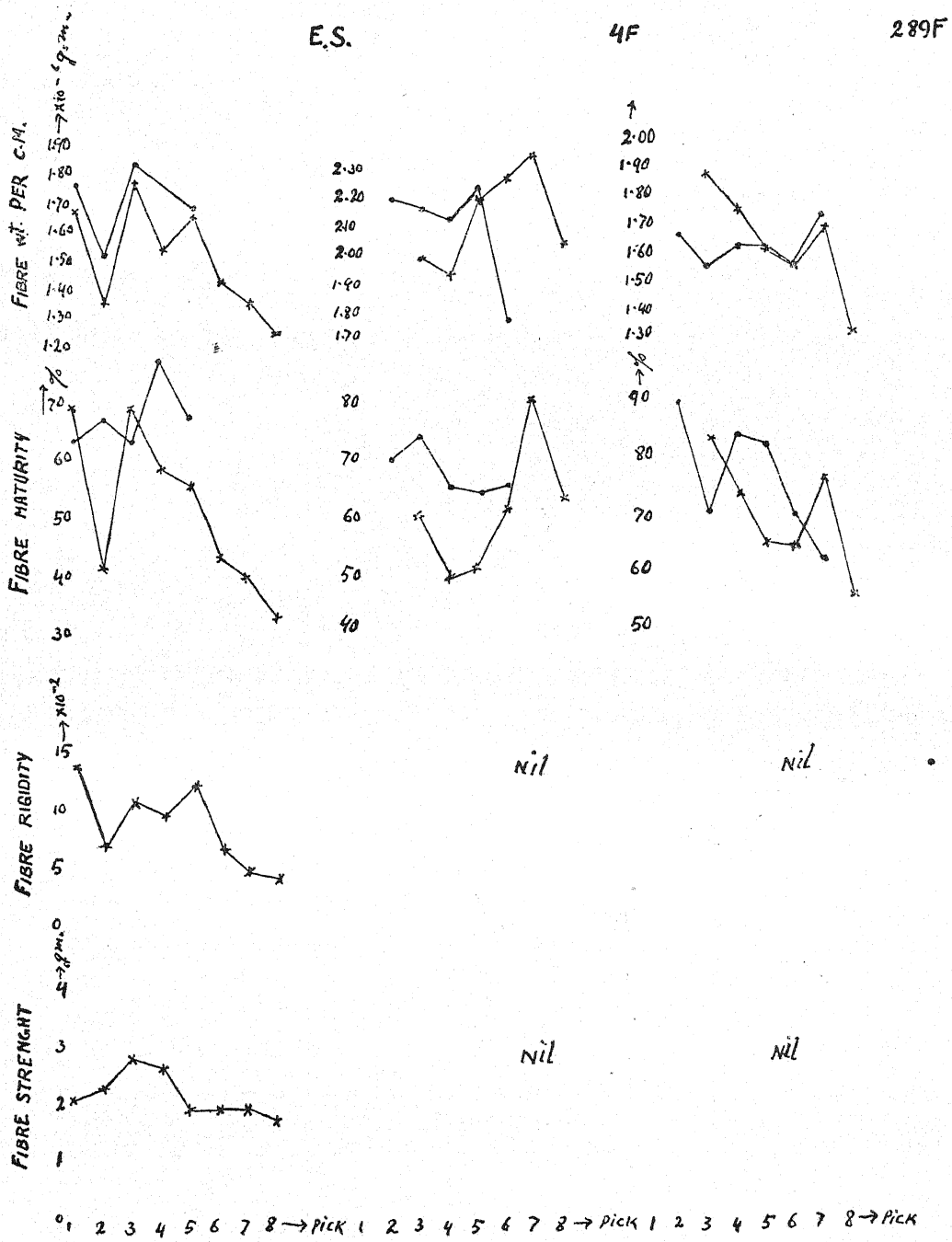

E.S.
4F
289F
FIBRE WT. PER C.M.
→ X10⁻⁶ gms.
FIBRE MATURITY
%
FIBRE RIGIDITY
→ X10⁻²
FIBRE STRENGHT
→ gms.
Nil
Nil
Nil
Nil
1 2 3 4 5 6 7 8 → PICK
1930-31
1931-32

Fig. 3.

(2) *Fibre length.*—In the same figure (Fig. 2) it is seen that for 1930-31 average fibre length decreased considerably in the case of Early Strain and 289 F towards the end of the season whereas in the case of 4 F it showed an increase. Due to mildness of this season, however, the picking for the present experiments were over rather early and consequently the number of pickings was also small. It is also observable that there was some fluctuation in the average length for Early Strain and 289 F during the beginning of the season while for 4 F this character was constant.

TABLE IV.

Modal length, fibre length irregularity and number of fibres of different lengths per seed.

Pick No.	1930-31			1931-32										
	P.-A. 4 F			Early Strain				P.-A. 4 F			P. A. 289 F.			
	Mean Modal length (cm.)	Fibre length irregularity (percentage)	Number of fibres of different lengths per seed $= \frac{1}{100} \times \frac{L}{f} \Sigma \frac{w}{l}$	Mean modal length (cm.)	Fibre length irregularity (percentage)	Number of fibres of different lengths per seed $= \frac{1}{100} \times \frac{L}{f} \Sigma \frac{w}{l}$	Fibre strength per unit fibre weight or intrinsic strength ($\times 10^{-6}$-grm.)	Mean modal length (cm.)	Fibre length irregularity (percentage)	Number of fibres of different lengths per seed $= \frac{1}{100} \times \frac{L}{f} \Sigma \frac{w}{l}$	Mean modal length (cm.)	Fibre length irregularity (percentage)	Number of fibres of different lengths per seed $= \frac{1}{100} \times \frac{L}{f} \Sigma \frac{w}{l}$
1	..	..	..	2·55	15·8	8784	0·52	..	..	..	..	..	..
2	2·09	9·1	5911	2·75	15·0	11248	0·62	..	..	..	..	..	..
3	2·18	8·6	9346	2·66	12·7	8667	0·60	1·87	9·2	8160	2·70	5·9	8456
4	2·15	8·4	7918	3·04	23·4	9438	0·63	1·93	7·9	7134	2·73	8·5	7800
5	2·23	7·8	7539	2·78	18·1	9462	0·43	1·93	5·2	6612	2·87	7·3	7221
6	2·24	5·5	7915	2·66	20·0	9095	0·53	1·88	6·2	7280	2·73	9·7	7992
7	..	..	..	2·72	24·4	8949	0·57	1·59	4·5	7272	2·76	9·2	7392
8	..	..	..	2·51	24·1	10854	0·58	1·71	9·8	8337	2·80	13·8	8755

In 1931-32, for all the varieties, the average fibre length was small at the beginning of the season, rose to a maximum during the middle part of the season and ultimately fell towards the end.

It is also found from Table IV that for 1931-32 the modal length or the length of fibres which occur most frequently in the sample, was low at the beginning, rose to a maximum and again fell at the end of the season. The fibre length irregularity or the percentage of fibres of length smaller than the three-fourths of the modal length, for 1931-32, increased to a certain extent towards the end of the season. In the case of the earliest maturing variety (Early Strain) this increase was considerable, while in the case of the variety maturing very late (289 F) it was fairly rapid.

(3) Fibre maturity and fibre weight per cm.—From the graphical representation (Figs. 2 and 3) of the results, as stated in Table III, of a normal season like 1931-32, it is found that variation in the maturity of fibres was a direct cause of variation in the fibre weight per cm. Although this relationship was not perfectly exhibited in the case of the particularly mild season, 1930-31, as in the other case, yet the fact remains that generally the fibre weight per cm. rose or fell with fibre maturity. Referring to Table V, it is found that fibre maturity and fibre weight per unit length for 1931-32 are significantly correlated in two cases out of three. Considering the results for 1931-32 (as in this case the number of pickings was large enough to give a clear indication of variation), it was found that both fibre weight per cm. and fibre maturity seemed to deteriorate in the case of Early Strain as well as of 289 F as the season progressed. In the case of Early Strain there were of course some fluctuations in the beginning of the season while in the case of 289 F some fluctuations occurred most markedly towards the end. In the case of 4 F, however, both the characters appeared to fluctuate towards the beginning as well as the end of the season.

(4) Fibre weight per cm. and rigidity.—The rigidity results, determined for Early Strain (1931-32) alone, indicate a close relationship to fibre weight per cm. (Figs. 2 and 3). From Table VI also it is found that the coefficient of correlation between fibre rigidity and fibre weight per cm. or fibre maturity is significant.

It is quite likely that the short maturation periods of the earliest bolls may be under the circumstances, insufficient for the full elongation of the hairs. Consequently it is probable that the earliest bolls should not only give shorter fibres but also should show fluctuations, especially in the case of the earliest maturing variety, *viz.*, Early Strain, in fibre maturity, as well as in fibre weight per cm. and fibre rigidity, which characters are the direct consequence of fibre maturity or wall thickening. Again towards the end of the fruiting season, due to a reduction in nutrients

as well as unfavourable weather conditions, it is very likely that there should be improperly developed fibres, both in length and in weight or wall thickness, in spite of long maturation periods. All these possibilities are fairly indicated by the present results (Figs. 2 and 3.)

(5) *Fibre breaking strength.*—Although it was generally expected that fibre breaking strength should very closely follow fibre maturity or fibre weight per cm. the results in the case of Early Strain(1931-32) showed (Figs. 2 and 3) that fibre breaking strength did not exclusively depend on fibre maturity. This is probably due to the fact that the breaking strength depends on the strength of the weakest point in the fibre which may not be proportionately raised by the wall thickening or fibre maturity.

The intrinsic strength or the strength per unit fibre weight calculated in Table IV was observed to change rather irregularly during the season.

(6) *Number of fibres of different lengths per seed.*—With regard to the number of fibres of different lengths per seed (Table IV) it was invariably found in the case of all the three varieties that this number was much higher for the last picking than for the pickings during the best part of the season. At the end of the season cotton fibres per seed seemed to gain in number while they lost in weight.

TABLE V.

Correlation coefficients, r, *by the product moment method together with significance calculated by Fishers'* t-P *criterion.*

Characters correlated	Early Strain					4 F					289 F				
	r*	n§	t¶	P	Remarks	r*	n§	t¶	P	Remarks	r*	n§	t¶	P	Remarks
(1) Seed index and average fibre length.	+0·14	6	0·3465	>0·7	Non-significant	—0·08	4	0·016	>0·9	Non-significant.	+0·16	4	0·324	>0·7	Non-significant
(2) Seed index and percentage of fully ripe hairs.	+0·14	6	0·3465	>0·7	Non-significant	+0·10	4	0·201	>0·8	Non-significant.	+0·87	4	3·551	<0·05	Doubtfully significant.
(3) Seed index and fibre strength.	+0·28	6	0·7146	>0·4	Non-significant	...	...	...	...	...	...	...	...	...	...
(4) Average fibre length and fibre strength.	+0·70	6	2·402	>0·05	Non-significant	...	...	...	...	...	...	...	...	...	...
(5) Average fibre strength and fibre weight per cm.	+0·60	6	1·838	>0·05	Non-significant	...	...	...	...	...	...	..	...	...	...
(6) Fibre weight per cm. and percentage of fully ripe hairs.	+0·96	6	8·400	<0·01	Significant .	+0·53	4	1·250	>0·2	Non-significant	+0·96	4	5·333	<0·01	Significant.
(7) Fibre weight per cm. and fibre rigidity.	+0·92	6	5·78	<0·01	Significant .	...	...	...	...	...	...	...	...	...	...
(8) Percentage of fully ripe hairs and fibre rigidity.	+0·92	6	5·78	<0·01	Significant .	...	...	...	...	...	...	...	...	...	...
(9(Percentage of fully ripe hairs and mean maximum air temperature.	+0·72	6	2·567	<0·05	Doubtfully significant.	—0·54	4	1·283	>0·2	Non-significant	+0·62	4	1·850	>0·10	Non-significant.
(10) Percentage of fully ripe hairs and mean hours of sunshine.	+0·77	6	2·957	<0·05	Doubtfully significant.	...	...	...	...	...	...	...	...	...	...

* $r = \dfrac{\Sigma (x\,y)}{\sqrt{\Sigma (x^2)\,\Sigma (y^2)}}$ where x, y, are respective deviations from mean.

§ n = actual number of pairs of observations on which correlation coefficient, r, is based *minus* 2.

¶ $t = \dfrac{r}{\sqrt{1-r^2}}\sqrt{n}$

TABLE VI.

Effect of pickings on certain fibre properties (By Fishers' method of Analysis of Variance).

Varieties analysed	Character	Degrees of freedom (n_1)	Degrees of freedom for random variation (n_2) etc.	'z' (Calculated)	'z' from Fishers table. For $P = 0{\cdot}01$	'z' from Fishers table. For $P = 0{\cdot}05$	Remarks
I. E. S. (Omitting the 1st and the 2nd pickings) 4 F and 259 F.	G. O. T.	5	10	0·689	0·865	0·601	Doubtfully significant
	Average fibre length.	5	10	0·738	0·865	0·601	Ditto.
II. 4 F. and 289 F.	Percentage of fully ripe hairs.	5	5	0·155	0·197	0·810	Non-significant.
	Fibre weight per cm.	5	5	Negative.	1·197	0·810	
III. Early Strain (Omitting the 1st and the 2nd pickings), and 289F.	Average fibre length.	5	5	0·399	1·197	0·810	Ditto.
	Fibre weight per cm.	5	5	1·269	1·197	0·810	Significant.

(7) *Pickings and quality.*—The intricate "interrelationship" of meteorological factors in their effect on plant nutrition and development has been stressed by Blackman [1929]. It is therefore true that no clear correlation can be expected between any fibre character and any one meteorological factor. Comparing Table III with Appendix II it is however, observable that there was a progressive deterioration of characters like maturity, fibre weight per cm. etc., in concordance with a progressive decrease of maximum air temperature simultaneously with a progressive decrease in the mean soil temperature gradient and an increase in the relative humidity observed at 8 hours.

Testing the data by Fisher's [1930] method of "Analysis of Variance" does not apparently give much encouraging result (Table VI) with regard to the effect of picking on the primary characters of cotton. Table VI indicates that the time of picking had little effect on fibre maturity or fibre weight per cm. of the late maturing varieties, and considering all the three varieties the effect of picking on average fibre length was doubtful. If however, only Early Strain and 289 F are considered, since 4F is different from them either morphologically or physiologically, a significant effect of picking is observed in the case of fibre weight per cm. although the effect on the average fibre length is non-significant. In the case of these two cottons (Early Strain and 289F) for the application of the method of analysis of variance, as the first two picks of Early Strain had to be disregarded, the fibre weight per cm. almost progressively decreased for both these cottons during the part of the season under consideration. The variation in the torsional rigidity values between any two picks, can be tested by the method of standard error of

difference (Table VII). It is clear from these results that although apparently the time of picking seems to induce some variation in fibre rigidity, the torsional rigidities between consecutive pickings do not, generally, vary significantly.

TABLE VII.

Significance of fibre rigidity difference between any two consecutive pickings.

Early Strain (1931-32).

Pick No.	Mean fibre rigidity ($\times 10^{-2}$ dynes cm^2)	Difference in fibre rigidity between consecutive pickings	3×S. E.	Remarks
1	13·31			
		6·71	3·15	Significant.
2	6·60			
		3·81	3·30	Significant.
3	10·41			
		1·08	3·63	Non-significant.
4	9·33			
		2·37	3·87	Non-significant.
5	11·70			
		5·56	3·42	Significant.
6	6·14			
		1·80	2·55	Non-significant.
7	4·34			
		0·76	1·89	Non-significant.
8	3·58			

VI. CONCLUSIONS.

The most important conclusions from the present experiments are as follows :—

(1) Although the present data is not suitable for the satisfactory investigation of the relationship between fibre properties and any of meteorological factors, yet trends, such as the progressive deterioration of some of the fibre characters (maturity and fibre weight) with progressive decrease of soil and air temperatures can be seen from an examination of Table III and Appendix II.

(2) There is no correlation between seed index and the average fibre length although Balls says that seed index should generally fluctuate with lint length.

(3) The present results indeed agree with Kottur's in so far as there is no correlation between the average fibre length and ginning outturn.

(4) There is a significant correlation between fibre maturity and fibre weight per cm. during the different pickings in the course of a season.

(5) The average number of fibres of different lengths per seed (as given in Table IV) is considerably greater at the beginning and end than at other times of the season.

(6) With particular reference to the Early Strain, it is found :

(i) That there is no significant correlation between fibre strength and fibre weight per unit length ;

(ii) That there is high correlation between torsional rigidity of fibres and either fibre maturity or fibre weight per cm. ;

(iii) That there is a slight tendency among the fibre strength values to progressively deteriorate towards the latter part of the season in a similar way to the values of fibre maturity or fibre weight per cm. ;

(iv) That apparently, the torsional rigidity of fibres diminishes progressively as the season advances—the difference between any two pickings, provided the pickings are sufficiently apart, is generally significant (in the case of the present experiments the difference between the consecutive pickings was insignificant, however, during the middle and the extreme end of the season) ; and finally,

(v) That the percentage of immature fibres definitely increases towards the end of the season.

This last conclusion (v) with regard to the Early Strain, holds for 289 F also, while for 4 F, this percentage is high towards the middle of the season.

(7) As a general effect of picking on fibre characters it may be stated that the early maturing variety of the Punjab-American cottons grown at Lyallpur seems to be more liable to variation in the important fibre characters than the late maturing varieties.

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Appendix I.

Period of boll maturation (days) for 1931-32.

Pick No.	Date of picking	Mean date between two consecutive picks	Average maturation period (days) for the bolls opening on or about the mean date		
			Early Strain	4 F	289 F
1	Date of commencement 10th September 1931*. to 24th September 1931.	17th September 1931.	39	..	..
2	24th September 1931. to 8th October 1931.	1st October 1931.	34	..	..
3	8th October 1931. to 24th October 1931.	16th October 1931.	35	40	45
4	24th October 1931. to 5th November 1931.	30th October 1931.	45	44	40
5	5th November 1931. to 19th November 1931.	12th November 1931.	53	52	53
6	19th November 1931. to 3rd December 1931.	26th November 1931.	60	60	61
7	3rd December 1931. to 17th December 1931.	10th December 1931.	65	60	63
8	17th December 1931. to 2nd January 1932.	25th December 1931.	67	70	71

* On 10th September 1931 there were no open bolls.

APPENDIX II.

Average meteorological data during the fortnight between two picks.

Fortnight preceding pick No.	1930-31					1931-32						
	Average mean daily soil temperature (°F.) at 30 cm.	Average air temperature (°F.)		Average relative humidity at 8 hours (per cent.)	Average sunshine (hours)	Average mean daily soil temperature (°F.)		Soil temperature difference. (°F.)	Average air temperature (°F)		Average relative humidity at 8 hours (per cent.)	Average sunshine (hours)
		Maximum	Minimum			At 5 cm.	At 30 cm.		Maximum	Minimum		
1	86·7	95·3	75·4	78·5	10·5	92·1	85·5	6·6	97·4	72·2	75·9	10·7
2	84·6	96·4	61·1	74·7	10·7	92·3	81·7	10·6	91·7	72·9	78·6	9·0
3	81·1	94·3	60·8	67·1	10·0	79·2	77·0	2·2	89·6	62·8	79·6	9·3
4	70·5	83·8	51·6	84·4	8·6	69·6	66·7	2·9	83·5	51·9	74·2	9·6
5	66·0	82·0	44·6	77·9	8·0	61·9	61·7	0·2	80·8	47·7	76·4	8·6
6	62·1	77·9	44·7	89·4	7·3	59·9	58·8	1·1	79·5	46·1	90·1	8·2
7	56·3	74·9	38·6	85·2	7·0	55·6	55·0	0·6	73·5	40·5	98·5	7·3
8	—	—	—	—	—	50·5	50·0	0·5	70·9	35·0	93·8	7·7

DETERMINATION OF NITROGEN IN SOILS, III.

FURTHER OBSERVATIONS ON THE PROTECTIVE ACTION OF SILICA AND THEIR BEARING ON THE ESTIMATION OF NITROGEN IN SUBSTANCES WHICH ARE ADMIXED WITH SOIL OR ARE OTHERWISE RICH IN SILICA.

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(With one text-figure)

It has been shown in the previous communication [Sreenivasan and Subrahmanyan, 1933] that incompleteness of digestion of soil by the present official methods is due to formation of protective coats of silica around undigested soil particles. Evidence has also been obtained to show that the reaction between hot, concentrated sulphuric acid and soil silicates results in the immediate formation of silica which covers the unattacked soil particles and thus protects them against the action of the acid. Since silicates form a large part of the clay fractions of soils and since clay soils offer more resistance to digestion than sandy ones, it appeared probable, therefore, that increase in the proportion of clay in a soil would lead to corresponding fall in the efficiency of digestion. The previous observations also suggested that accurate estimation of nitrogen in a number of organic substances containing soil or silicates admixed with them or silicon in organic combination may be adversely affected in the same manner as that of soils. The present investigation was therefore undertaken with a view to throwing some light on the above and related problems.

Estimation of nitrogen in clay from different soils.—The clay and other finer fractions from three different soils were separated in the usual way. They were then dried and their nitrogen contents compared with those of the soils from which they were derived, according to both 'dry' and 'wet' methods (Table I).—

TABLE I.

Mode of digestion	Nitrogen in parts per millon (averages)						Standard Error
	Light soil—Bangalore		Heavy soil—Bangalore		Heavy black soil—Nagpur		
	Soil	Clay	Soil	Clay	Soil	Clay	
'Dry, (Official) .	211·4	556·3	900·7	1663	648·2	1016	±2·0
'Wet, (Overnight)	219·6	580·9	934·0	1734	745.7	1175	±0·7

The incompleteness of digestion by the 'dry' method is more prominently seen in the case of clay fractions than in those of whole soils.

Effect of increasing the clay content of a soil.—To 5-grm. lots of a specimen of soil from Nandyal, 0·54, 1·08 and 2·70 grms. respectively of clay fractionated from a different soil were added and the nitrogen contents of the mixtures determined (Table II).

TABLE II.

Mode of digestion	Nitrogen in parts per million (averages)					
	Soil (control)	Clay (control)	Soil + clay (0·54 grm.)	Soil + clay (1·08 grm.)	Soil + clay (2·7 grms.)	Standard Error
'Dry' (Official) . . .	248·5	1663	384·4	489·0	734·7	±2·6
'Wet' (Overnight) . .	306·1	1734	446·6	556·5	805·6	±0·8

In addition to giving low values, increase in the proportion of clay rendered digestion by the 'dry' method very difficult to carry out. On the other hand, 'wet' digestion proceeded smoothly in all the cases.

Effect of addition of silicious earths.—The above experiment was repeated adding acid-washed specimens of Kaolin or Kieselguhr in place of fractionated clay (Table III). The added materials contained no nitrogen.

TABLE III.

Mode of digestion	Nitrogen in parts per million (averages)						
	Soil alone (control)	Soil + Kaolin (1 grm.)	Soil + Kaolin (3 grms.)	Soil + Kaolin (5 grms.)	Soil + Kieselguhr (1 grm.)	Soil + Kieselguhr (5 grms.)	Standard Error
'Dry' (Official) .	942·9	934·0	929·6	909·7	960·6	925·2	±1·6
'W' (Overnight)	982·1	980·5	982·6	978·4	982·6	980·5	±0·9

The results show that the added materials have appreciably affected the accuracy of estimation by the 'dry' method in spite of the previous treatment

which would have rendered them less efficient than they might otherwise have been.

Estimation of nitrogen added to a silicious material.—A specimen of garden soil (500 grms.) was treated with (1,000 c.c.) 4 per cent. sodium hydroxide solution and the suspension thus obtained allowed to stand for some time. The clear, supernatant liquid containing humus in solution was then filtered and different quantities of the filtrate added to 5-grms. lots of a specimen of untreated Kaolin obtained locally. Sufficient acid was added to neutralize excess of alkali and the pastes containing neutral humus slowly evaporated to dryness. The nitrogen contents of the dried preparations were determined by 'dry' and 'wet' methods (Table IV).

TABLE IV.

Mode of digestion	Nitrogen in parts per million (averages)					
	Kaolin (control)	Kaolin (5 grms.) + soil extract (10 c.c.)		Kaolin (5 grms.) + soil extract (20 c.c.)		Standard Error
		Expected	Found	Expected	Found	
'Dry' (Official) . . .	75·4	936·3	909·5	1788	1759	±3·1
'Wet' (Overnight) . .	77·7	940·6	934·0	1793	1786	±0·2

The presence of silicious matter renders the estimate of nitrogen by the 'dry' method less accurate than that by the 'wet' method.

Study of nitrogen transformations in soils.—With a view to assessing the relative efficacies of the two methods in the study of nitrogen changes in soils, some experiments were carried out adding 0·399 grm. of dried blood (corresponding to 50 mgrms. of nitrogen) to 100-grms. lots of a specimen of soil and determining the nitrogen contents at stated intervals. One set of specimens were moistened with water and maintained at 60 per cent. saturation, while the others were treated with 100 c.c. each of distilled water and maintained in the waterlogged condition throughout the period of observation. At the time of sampling, the specimens were just acidified to avoid loss of ammonia through volatilization and then dried first over water bath and then in air at the room temperature. The dried specimens were powdered to pass the 30-mesh sieve and the nitrogen contents estimated (Table V).

TABLE V.

Treatment	Method of digestion	Nitrogen in parts per million (averages)							
		Time in days							Standard Error
		0	8	15	22	29	57	78	
Maintained at 60 per cent. saturation	'Dry' .	711	689	669	655	645	617	595	±0·2
	'Wet' .	730	724	700	689	677	659	626	Small
Waterlogged . .	'Dry' .	711	675	636	589	541	464	422	±1·2
	'Wet' .	730	714	677	626	589	523	489	Small

The difference between the values obtained by the two methods becomes increasingly prominent with time. This is strikingly so in the case of the waterlogged specimens in which the apparent loss of nitrogen on the 78th day is 16 per cent. more according to the 'dry' method than by the 'wet' one. The results thus show clearly that the present official methods are not reliable for the accurate study of nitrogen transformations in soils.

The decreasing efficiency of digestion by the 'dry' method in the case of the waterlogged specimens (Fig. 1) would suggest that the soil had undergone some change that led to such a result. Since previous observations had shown that increase in the silicate content of a soi leads to corresponding fall in the efficiency of digestion by the 'dry' method, it appeared probable that waterlogging, as also the presence of fermentable organic matter, had led to a similar condition. Indeed, the formation of organic acids [Subrahmanyan, 1929] and their reaction with the mineral matter of the soil would point to such a conclusion. Further work is in progress to throw more light on this aspect of the problem.

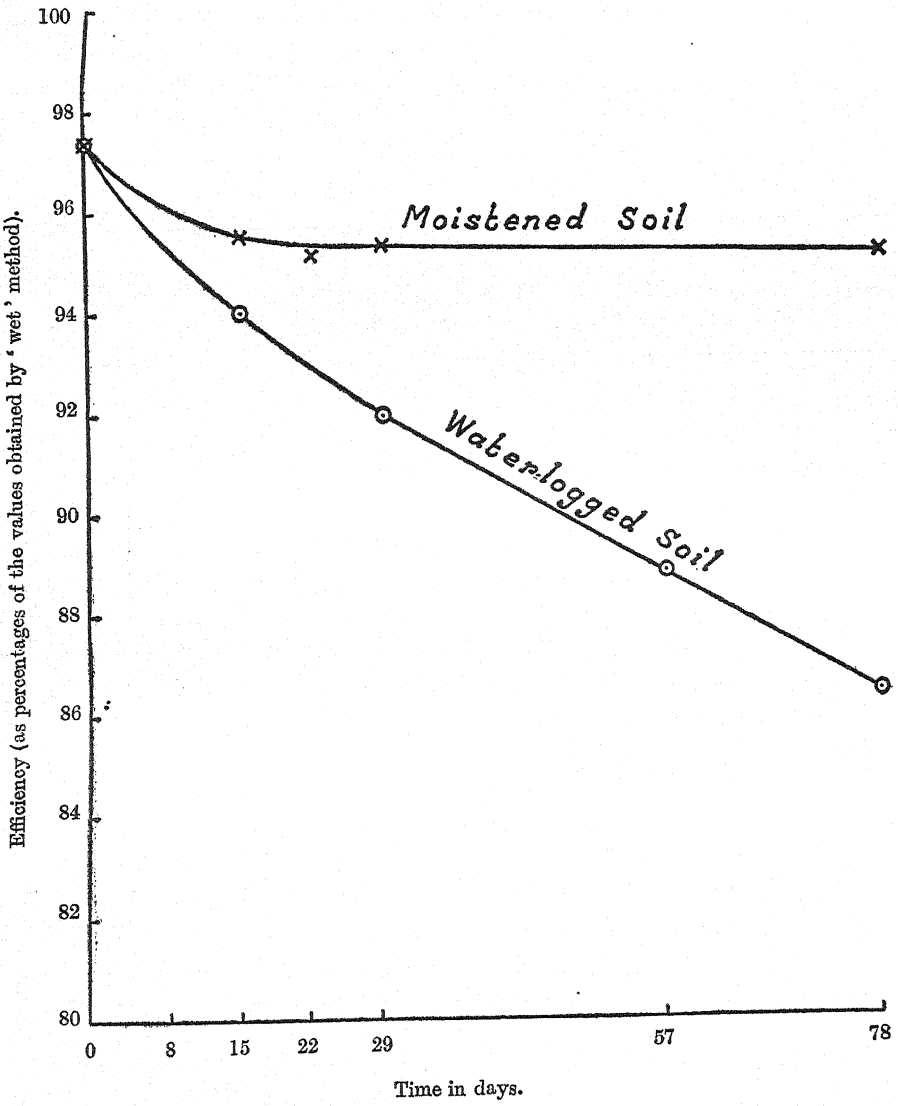


Fig. 1.—Efficiency of digestion by 'dry' method.

Estimation of nitrogen in plant materials.—With a view to determining whether the protective action of silica is also to be observed in the case of plant materials which are naturally rich in silicon, a number of determinations were carried out (Table VI.)—

TABLE VI.

Method of digestion	Nitrogen as parts per million (averages)			
	Paddy husk	Kusa grass	Elephant grass	Standard Error
'Dry' . . .	5,790 (0·579)	9,360 (0·936)	23,550 (2·355)	±1·3
'Wet' . . .	5,850 (0·585)	9,400 (0·940)	23,650 (2·365)	±1·2

The bracketted figures give the percentages on dry weight.

There is some difference between the values obtained by the two methods, though, on the percentage basis, it is almost negligible. There is no evidence of any protective action by silica but in view of the more efficient digestion obtained by the 'wet' method, it would appear to be desirable to adopt it in preference to the present official methods.

Determination of nitrogen in manures.—A number of organic manures are obtained admixed with varying quantities of soil or metallic silicates. Since it appeared probable that the latter may interfere with the accurate estimate of nitrogen in such substances, a few representative specimens were analysed by the two methods (Table VII.)—

TABLE VII.

Method of digestion	Nitrogen in parts per million (averages)					
	Seed cake (control)	Activated sludge	Precipitated sludge (with alum)	Compost from town refuse	Farmyard manure (inferior quality)	Standard Error
'Dry' (Official) . . .	26,640	33,880	29,010	12,520	12,870	±2·9
'Wet' (Overnight) . .	26,600	34,340	29,550	12,700	13,150	±1·1

With the exception of the seed cake which contained no silicate, all the others showed discordance between the values obtained by the two methods. The difference may not be considerable when the percentages are taken into consideration, but are of importance in any study relating to nitrogen transformations undergone by such materials either on storage or when applied to soil. It would therefore be desirable to adopt the 'wet' method for the accurate estimation of nitrogen in such materials.

SUMMARY.

1. Further evidence has been adduced to show that incompleteness of digestion by the present official methods of estimating nitrogen is due to the presence of silicates, particularly those of the clay fraction of the soil. Pre-treatment with water eliminates the protective action of silica and thus ensures complete digestion.

2. The method of 'dry' digestion is not suitable for the study of nitrogen changes in soils because it becomes increasingly inaccurate with time. There is evidence to show that this is the result of changes in the silicate content of the soil.

3. The protective action of silica is not noticeable in the case of plant materials. On the other hand, dry digestion of organic manures containing soil or silicates yield discordant results thereby showing that such forms should be 'wet' digested to obtain accurate estimates of nitrogen contained in them.

The author's thanks are due to Dr. V. Subrahmanyan for his interest in the progress of the work and helpful criticism.

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THE METHOD OF 'COVARIANCE' APPLICABLE TO THE UTILIZATION OF THE PREVIOUS CROP RECORDS FOR JUDGING THE IMPROVED PRECISION OF EXPERIMENTS.

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INTRODUCTION.

Fisher [1932] has shown that the precision of an experiment particularly on perennial crops like tea, can be considerably increased with a knowledge of yields from different plots subjected to the same treatment for a period prior to the experiment. Further, he has shown that if such prior records are available they may be utilized to judge the condition of the soil and to deduce a suitable design for subsequent experiments. The example of tea yields which Fisher [1932] has selected for illustrating his statistical method of ' covariance ' brings out an increase in accuracy in the experiment by nearly six times with the use of the data of preliminary yields. Sanders [1930] working on the results of uniformity trials on cereals at Aarslew (Denmark) during the years 1907-1911 examined whether the soil variations in the experimental plots are sufficiently constant from year to year to give useful corrections to the experimental yields from the yields during the preliminary periods and found that in one field the precision of the experiment was increased by 150 per cent. by utilizing the previous records, but that in another field the plots showed no constancy in yield and consequently previous uniformity trials did not give any assistance. Eden in his ' studies in the yield of tea ' [1931] shows that the application of the method of covariance (explained below) for correcting experimental yields on the basis of the previous cropping gives more satisfactory results in the case of perennial crops like ' tea ' than in the case of annual crops. Fisher [1932] does not recommend in general the cropping of the experimental plots in the year previous to the experiment as it involves double the labour of the experiment and at least a year's delay before the result is made available. But the fundamental point for consideration is whether, if such preliminary records are available, they cannot be utilized for designing new experiments in the same set of plots or for judging the precision of the experiment on the basis of the preliminary yields. The author's visit to the Tocklai Tea Experimental Station during December 1933 convinced him that by using preliminary yields in the case of manurial experiments on tea, an improvement in precision in the

experiment is definitely brought out and that they can be utilized for designing improved lay-out for future experiments.

DESIGN OF NEW EXPERIMENTS ON THE BASIS OF PRELIMINARY YIELDS.

A common method that is advocated for utilizing the preliminary yields for the design of a new experiment, is ' the equalization of plot yields ' in an entire field, that is to say, to choose sets of plots in the whole field for different treatments giving the same (or almost the same) preliminary yields for different sets. If for example 12 plots in a field have to be distributed between two treatments, A and B (*i. e.* with six replications for each treatment), to eliminate the errors caused by soil heterogeneity as affecting the treatment effects, we select two sets of six plots such that the total preliminary yield for each of the sets is the same (or almost the same). In this way we try to eliminate the error caused by the soil variation, for effecting an equitable comparison between yields resulting from several treatments. But the defect in the method is that such equalization as is possible is bound to be the inexact, and besides, there is a possibility of any number of such ' equal ' sets being available without any clear indication as to which set or sets the experimenter has to choose.

This method of ' equalization ', moreover presupposes that the relative inherent productivity of the plots during the preliminary and experimental years remains the same. Mathematically, we assume in such a case that $x—y=x^1—y^1$ where x, y, are the yields of any two plots in the preliminary period, and x^1, y^1, their yields in the experimental year due in each case to fertility alone ; or if there should be any correlation in fertility between the neighbouring plots we assume that the same correlative effect is kept up in both the periods. But the influence of ' season ' connot always be overlooked, unless there is reason to suppose that the relative effects of season on soils of different fertility during the preliminary and experimental periods remain the same. Further, such ' equalization ' in an entire field cannot take into account broad fertility differences between one part of the field and another, as in the case of ' randomised block arrangement ' or ' Latin Square ' where it is possible to eliminate those differences for a justifiable comparison of the treatment effects. Besides, a valid estimate of ' chance error ' is possible only if the elimination of variances due to systematic causes can be secured, and in the method of ' equalization ' of plots as ordinarily advocated, such a valid estimate may not be possible as treatments may be distributed without reference to fertility gradient.

An improvement in the above method of ' equalization ' of plots (*i. e.* choosing sets of plots of equal yields in an entire field) is to combine Fisher's method of block distribution with that of ' equalization ' of plots. By this improved

method, the experimental area is first divided into blocks to mark off the fertility gradient, and then sets of plots are chosen from the several blocks (one plot from each block to correspond to each treatment during the experimental year) such that the sum total of yields for different sets during the preliminary period is the same. This improved method, though recognising broad fertility differences in the experimental area and thus removing in a way the defect pointed out in the previous paragraph, has still the disadvantage that it may not always be possible to choose such 'equal' sets from several blocks, apart from the sacrifice, which it involves, of the advantages arising from randomised block arrangement or 'Latin Square' in working out the analysis of variance. The experience in Tocklai Experimental Station has however been that in the case of a perennial crop like tea it has been found possible to choose such sets based upon data of yields during the preliminary period, reducing the standard error of the experiment to even so low as 2 per cent. If it should then be possible to have such sets (*i. e.* a combination of block arrangement and 'equalised' sets) it seems desirable to have a lay-out on the above lines. On this aspect of the question Fisher and Wishart [1930] say, 'It is often felt, when first a randomised arrangement is compared with one in which the plots are arranged in a regular or systematic order that the latter has an advantage in that, the sets of plots treated alike being more regularly spread over the whole area, the errors in the comparisons between treatments will for this reason be reduced. This may often be really the case; what should be noted however, is that, as the arrangement of our calculations in the form of an analysis of variance makes clear, any 'improvement' on the random arrangement which reduces the real errors of our comparisons will be accompanied automatically by an increase in our estimate of error, a definite portion of the variance being simply transferred from one item to the other. Consequently, the skill and judgment devoted to obtaining plot arrangements which involve errors between the comparisons less than those of random arrangements may indeed make the experiment really better, but at the expense of making it seem worse. In the contrary case, if the experimenter were so unlucky as to his real errors, his estimate of error would be diminished. In either case the validity of his estimate of error is vitiated and it is impossible to be sure whether an over-estimate or an under-estimate has been obtained'.

If however, with reference to the preliminary data (subjected to the same treatment) we can analyse separately variances due to blocks (rows or columns) and randomness, the significance or otherwise of block variance (or variance of rows or columns) as compared to random variance can give a correct perspective of how best the experimental plots should be arranged. From such an analysis of variance of preliminary yields, it is possible, for example, to know whether there is a signifi-

cant variance in yields in 'rows' and 'columns' or again whether 'rows' and 'columns' themselves significantly differ. If it so happens that 'rows' show significant increase over 'columns', then the block arrangement of plots along 'columns' should be preferred and *vice versa*. Or again if both 'rows' and 'columns' show very high variances as compared to randomness, the size of the block should be appreciably reduced and the number of replications correspondingly increased. Thus the analysis of data of preliminary yields into 'block' variance and 'random' variance can always indicate an improved method of lay-out.

Method of covariance.

Apart from any method of lay-out that is found equitable for designing new experiments based upon preliminary yields, the main statistical problem is how best we have to adjust the data of experimental yields on the basis of preliminary data, so that we may get an improved precision over that we would have got without the use of the preliminary yields. In other words how is the standard error deduced from the experiment to be corrected on the basis of the standard error of the pre-experimental data? Such a correction is obviously based on an assumption of a relationship between the yields of the same plot during the preliminary and experimental years. Assuming some simple relationship between these two yields, x and y,—say a linear regression of y and x—, then for a comparison of the adjusted yields on which the corrected standard error depends, we have only to calculate values of $y-bx$ for individual plots, where b is the coefficient of regression. An estimate for b is given by the ordinary formula, $\frac{\text{mean } \Sigma xy}{\text{mean } \Sigma x^2}$ where x and y are measured from their respective means. The mean product of deviations of two variates is termed 'Covariance' in analogy with the term 'variance' applied to a single variate. The calculation of Σxy is a simple matter as only corresponding x and y (*i. e.*, corresponding figures taken from tables of preliminary and experimental yields) have to be multiplied and totalled up; b is then the ratio of the mean of this total to mean 'sum of squares' calculated for x. With the evaluation of b the next step is to get the analysis of adjusted yields, which is merely the analysis of $(y-bx)$ values for the individual plots. $(y-bx)^2$ being $= b^2x^2-2bxy+y^2$, $(y-bx)$ table is then evaluated, on the basis of the values of b, x and y. The standard error deduced from $(y-bx)$ table is then the corrected standard error.

Statistical tables for the evaluation of the corrected standard error.

Based on the above explanation, the tables necessary for working out the corrected standard error will be as follows. During the preliminary period hypothetical treatments have been assumed in analogy with the treatments during the experi-

mental period, so that the number of degrees of freedom for 'error' may be the same in Tables I (*c*), II (*c*), III (*b*) and IV (*b*). (Assume 4 treatments and 6 blocks).

TABLE I (*a*) (*x*-TABLE).

Preliminary yields (i. e., *subjected to the same treatment in the year or years previous to the experiment*).

	Hypothetical treatment				Total
	A	B	C	D	
Block I					
„ II					
....					
....					
....					
VI					
Total .					Grand total

Average :—

(Construct a subsidiary table giving the deviations from the average.)

TABLE I (*b*) (x^2-TABLE).

Squares of deviations from the average.

	A	B	C	D	Sum of squares
Block I . . .					
„ II					
....					
....					
....					
VI					
Total .					Grand total

TABLE I (*c*).

Analysis of variance of preliminary yields.

	Degrees of freedom	Sum of squares	Mean square
(1) Blocks	5		
(2) Hypothetical treatments . .	3		
(3) Error	15		
Total .	23		

TABLE II (*a*) (*y*-TABLE).

Experimental yields (subjected to several treatments).

	Treatment				Total
	A	B	C	D	
Block I					
„ II					
....					
....					
....					
VI					
Total .					Grand total

Average :—

(Construct a subsidiary table giving the deviations from the average.)

TABLE II (*b*), (y^2-TABLE).

(*Squares of deviations from the average.*)

Treatment.

	A	B	C	D	Sum of squares
Block I . . .					
II . . .					
....					
....					
....					
VI . . .					
Total .					Grand total

TABLE II (*c*).

Analysis of variance of experimental yields.

	Degrees of freedom	Sum of squares	Mean square
(1) Blocks	5	....	....
(2) Treatments . . .	3	....	....
(3) Error	15	....	....
Total .	23	....	....

TABLE III (*a*), (*xy*-TABLE).

Multiply corresponding values of x *and* y *in subsidiary tables under I* (a) *and II* (a).

Treatments.

	A	B	C	D	Total
Block I . . .					
II . . .					
.....					
.....					
....					
VI . . .					
Total .					Grand total

TABLE III (*b*).

Analysis of covariance.

	Degrees of freedom	Sum of products	Mean product
(1) Blocks	5	....	....
(2) Treatments . . .	3	.....	....
(3) Error	15	....	.....
Total .	23	....	....

TABLE IV (*a*).

[First calculate b (coefficient of regression) which is the ratio of mean 'Error xy product' to mean 'Error x^2' (taken from Tables III (*b*) and I (*a*)).]

(Construct a new table based upon Tables I (*c*), II (*c*) and III (*b*) by multiplying 'sums of squares' (or xy products) in the respective tables by b^2, 1 and $-2b$, respectively, and totalling them.)

TABLE IV (*b*).

(y-bx *analysis.*)

	Degrees of freedom	Sum of squares	Mean square
Blocks	5	....	....
Treatment	3	....	....
Error	*14	....	....
Total .	22	....	....

* One less than the number of degrees of freedom for 'error' in Table I (*c*) or III (*b*) to allow for the one adjustable constant in the linear regression formula.

The reduction of mean square for 'error' from that in Table II (*c*) to that in IV (*b*) shows the improvement in the precision of the experiment.

Illustration of 'covariance'.

The above method is applied to data of tea yields in Tocklai Experimental Station (details of plots and treatments are omitted). The plots for the treatments in the several blocks have been chosen on the basis of 'equalization' of plot yields during the preliminary period.

TABLE I (*a*), (*x*-TABLE).

Preliminary yields (subject to no treatment).

Plot yield.

	A	B	C	D	Total
Block I . . .	134	118	99	104	455
„ II . . .	133	105	138	142	518
„ III . . .	103	129	129	143	504
„ IV . . .	104	126	104	79	413
„ V . . .	143	127	142	127	539
„ VI . . .	109	113	111	127	460
Total .	726	718	723	722	2,889

Average about 120.

Subsidiary table (deviations from arbitrary average 120).

	A	B	C	D	Total
Block I . . .	14	—2	—21	—16	—25
„ II . . .	13	—15	18	22	38
„ III . . .	—17	9	9	23	24
„ IV . . .	—16	6	—16	—41	—67
„ V . . .	23	7	22	7	59
„ VI . . .	—11	—7	—9	7	—20
Total .	6	—2	3	2	9

TABLE I (*b*), (x^2-TABLE).

	A	B	C	D	Sum of squares
Block I . . .	196	4	441	256	
„ II . . .	169	225	324	484	
„ III . . .	289	81	81	529	
„ IV . . .	256	36	256	1,681	
„ V . . .	529	49	484	49	
„ VI . . .	121	49	81	49	
Total .	1,560	444	1,667	3,048	6,719

Correction for average $= \frac{9 \times 9}{24} = 3{\cdot}375$

Grand total $= 6{,}715{\cdot}625$

Block—'sum of squares.'

Deviation	Sum of squares
—25	625
38	1,444
24	576
—67	4,489
59	3,481
—20	400
	11,015
Divide by 4 (the number of plots in each block)	=2,753·75
Correction for average :—	3·375
Total block 'sum of squares' .	=2,750·375

TABLE 1 (*c*).

Analysis of variance of preliminary yields.

—	Degrees of freedom	Sum of squares	Mean square
(1) Blocks	5	2,750·375	550·75
(2) Hypothetical treatments . . .	3	5·458	1·82
(3) Error	15	3,959·792	263·99
Total .	23	6,715·625	291·98

TABLE II(*a*) (*y*-TABLE).

Experimental yields.

—	A	B	C	D	Total
Block I	135	110	98	117	460
„ II	136	107	145	175	563
„ III	102	125	138	181	546
„ IV	108	136	114	96	454
„ V	149	116	164	144	573
„ VI	110	110	120	152	492
Total	740	704	779	865	3,088

Subsidiary table (*deviation from arbitrary average 130*).

5	—20	—32	—13	—60
6	—23	15	45	43
—28	—5	8	51	26
—22	6	—16	—34	—66
19	—14	34	14	53
—20	—20	—10	22	—28
—40	—76	—1	85	—32

TABLE II (*b*), (y^2-TABLE)

Squares of deviation from the average

	A	B	C	D	Sum of squares
Block I	25	400	1024	169	
„ II	36	529	225	2025	
„ III	784	25	64	2601	
„ IV	484	36	256	1156	
„ V	361	196	1156	196	
„ VI	400	400	100	484	
Total	2090	1586	2825	6631	13132

Correction for average $= \frac{32^2}{24} = 42\cdot\dot{6}$

Grand total:—13089·3

Block sum of squares

Deviation	Sum of squares
—60	3600
43	1849
26	676
—66	4356
53	2809
—28	784
	14074
Divide by 4	3518·5
Correction for average	42·6̇
Block variance	3475·83

Treatment 'sum of squares'

Deviation	Sum of squares
—40	1600
—76	5776
—1	1
85	7225
	14602
Divide by 6	2433·6
Correction for average	42·6̇
Treatment variance	2391·0

TABLE II (*c*).

Analysis of variance of experimental yields

	Degrees of freedom	Sum of squares	Mean square
Blocks	5	3475·83	695·17
Treatments	3	2391·00	797·00
Error	15	7222·50	481·50
Total	23	13089·33	569·10

TABLE III (*a*) (*xy*-TABLE).

Experimental yields (deviations from arbitrary averages).

	x-Table (arbitrary average 120)					*y*-Table (arbitrary average 130)				
	A	B	C	D	Total	A	B	C	D	Total
Block I	14	—2	—21	—16	—25	5	—20	—32	—13	—60
„ II	13	—15	18	22	38	6	—23	15	45	43
„ III	—17	9	9	23	24	—28	—5	8	51	26
„ IV	—16	6	—16	—41	—67	—22	6	—16	—34	—66
„ V	23	7	22	7	59	19	—14	34	14	53
„ VI	—11	—7	—9	7	—20	—20	—20	—10	22	—28
Total	6	—2	3	2	9	—40	—76	—1	85	—32

	xy-Table				Total	Products for 'blocks'
Block I	70	40	672	208	990	1,500
„ II	78	345	270	990	1,683	1,634
„ III	476	—45	72	1,173	1,676	624
„ IV	352	36	256	1,394	2,038	4,422
„ V	437	—98	748	98	1,185	3,127
„ VI	220	140	90	154	604	560
Total	1,633	418	2,108	4,017	8,176	11,867

Correction for average $\frac{9 \times -32}{24} = -12$

Crude sum of products	= 8,176
Subtract correction	= —12
Total Σxy	8,188
Crude sum of block total	= 11,867
Divided by 4	= 2,966·75
Subtract correction	= —12
Σxy for blocks	= 2,978·75

TABLE III (*b*).

Analysis of covariance.

	Degrees of freedom	*xy* products	Mean product
(1) Blocks	5	2978·75	595·75
(2) Treatments	3	25·17	8·39
(3) Error	15	5184·08	343·60
Total	23	8188·00	356·00

TABLE IV(*a*).

b. (Coefficient of regression)$=\frac{5184\cdot08}{3965\cdot25}=1\cdot3092$.

(Note that since degrees of freedom for 'error' in Tables I(*c*) and III(*b*) are the same, it is convenient to take in this case the corresponding *xy* product direct rather than mean product.)

$b^2=1\cdot7140$; $-2b=-2\cdot6184$.

Sums of squares and products taken from Tables I(c), III(b) and II(c).

	Degrees of freedom	x^2	xy	y^2
Blocks	5	2750·375	2978·75	3475·83
Treatments	3	5·458	25·17	2391·00
Error	15	3959·792	5184·08	7222·50

TABLE IV(*b*).

Analysis of variance of adjusted yields (y—bx)

	Degrees of freedom	y^2	b^2x^2	$-2bxy$	$y^2+b^2x^2-2bxy$	Mean square
Block . .	5	3475·83	2750·37 × 1·714	—2978·75 × 2·6184	390·414	78·08
Treatments .	3	2391·00	5·46 × 1·714	—25·17 × 2·6184	2334·450	778·15
Error . .	14 (after allowing 1 degree for linear regression).	7222·50	3959·79 × 1·714	—5184·18 × 2·6184	435·588	31·11
Total .	22				3160·452	

Improvement in precision by using the preliminary data

$$=\frac{\text{Mean square for 'error' in Table II}(c)}{\text{Mean square for 'error' in Table IV}(b)}$$

$$=\frac{481\cdot5}{31\cdot11}=\text{about } 15\cdot5 \text{ times.}$$

Inferences from the analysis of the above data of 'tea yields'.

Table I(*c*) shows that the elimination of 'block variance' from preliminary data has reduced the variance due to error though by a small quantity only in this case, thus emphasising the need for marking off 'blocks' in an experimental field, and for superseding old experimental methods of apportionment of plots without reference to fertility gradient. Table II(*c*) shows that even after elimination of 'block' and 'treatment' variances considerable residual error remains; but nearly half the total 'sum of squares' has been eliminated. Table III(*b*) which analyses the 'covariance', shows that *xy* product for 'error' is appreciable, as must be the case in the present example as during the preliminary period there was only one uniform treatment (or no treatment), and as the effect of the experiment, at least one treatment (in this case treatment 'D'), has shown very high increases over others. Table IV(*b*) which analyses the adjusted yields after allowing for linear regression shows that by combining the 'preliminary' and 'experimental' analyses the standard error of the experiment has been considerably reduced. The improvement in precision is nearly 16 times what it would otherwise be by analysing the experimental data alone. Thus it is seen that where preliminary yields of experimental plots can be secured, it seems advantageous to explore them fully and make valid use of them. By using such data, not only is an 'improved' lay-out possible but there is a possibility of securing a 'greater' precision on which a more equitable comparison of 'treatments' should necessarily depend.

The author is indebted to Mr. P. H. Carpenter, Chief Scientific Officer, Indian Tea Association, for having invited the author to visit the Tocklai Experimental Station during winter 1933 when the principle of 'covariance' was discussed in its different aspects. Thanks are specially due to Mr. Carpenter for allowing his data to be used in this paper.

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STUDIES ON *CERCOSPORA INDICA*, N. SP., PARASITIC ON *CAJANUS INDICUS* SPRENG.

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(With Plates XXI-XXIII and four text-figures)

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I. Introduction.

A leaf-spot disease of *Cajanus indicus* caused by a species of *Cercospora* is very common in Bihar and the United Provinces, and differs in morphology

from *C. Cajani* Rangel and P. Henn. and *C. instabilis* Rangel previously recorded on this host from other countries, *i.e.*, St. Rio de Janeiro. Sio Paulo, Minas, Geraes. Brasiliae and Niteroy. Brasiliae respectively. Two strains of this species were isolated, one (CA)* from Allahabad and the other (CP)* from Pusa and were found to be strains of a new species hitherto undescribed. In addition to this, *C. dolichi* E. and E. which causes infection of *Dolichos Lablab* Linn. was also noticed to infect *Cajanus indicus*, especially when the two crops are grown side by side.

The present study deals with the parasitism of these two strains, their cultural characteristics on different artificial media, and their physiological response to various environmental conditions.

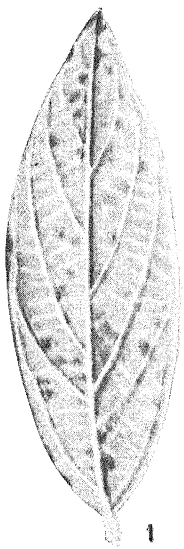
II. Morphology of the organism in nature.

The disease makes its first appearance on the under-surface of leaves as small light brown spots, 1-2 mm. in diameter. These spots are at first more or less roundish, but later on become irregular in outline, and occasionally several coalesce forming irregular areas as large as 15 mm. $\times$5 mm. Spots seldom, if ever, cross the midrib or the primary veins of the leaflets. The centre of these spots is dark brown and bear the fascile of conidiophores with conidia (Plate XXI, figs. 1-6). On older spots where conidiophores have ceased to form spores, infected areas become very thin and translucent. In advanced stages the whole of the leaf dries, curls and ultimately falls. The lesions on petioles are less common than on leaves, but more than on stems (Plate XXI, figs. 7 and 8). These are greyish black and run parallel to the long axis of the petiole.

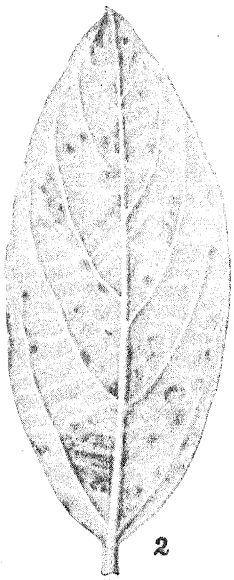
The mycelium of the fungus is both inter and intra-cellular, the hyphae collect in the air spaces under the stomata and form stromatic masses giving rise to conidiophores which emerge from the stoma. The fungus also forms creeping external mycelium on the surface of the leaf.

Conidiophores are light brown when young, dark brown when mature, and are found mostly on the under-surface of the leaf and come out in definite fascile arising from loose stromatic masses, found in the air spaces of the leaf (Plate XXI, figs. 1-3). Often they are covered with a series of geneculations marking the points of attachment with conidia (Plate XXII, figs. 4-6). Branched conidiophores are by no means rare. These vary in length and septation according to moisture conditions. Their size ranges from 28·0μ-168·0μ $\times$3·4μ-7·0μ and septation 2-13 with a slight constriction in some conidiophores just near the septa. Conidiophores or their pieces readily germinate in tap water in 10-12 hours at 25°C. The contents are highly granular due to the presence of refractive oil globules.

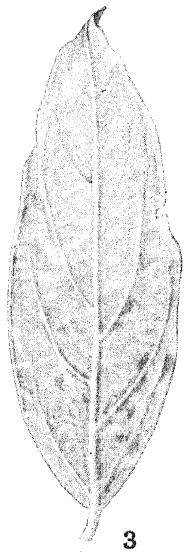
* For simplicity these strains will be referred to in the paper by these symbols.



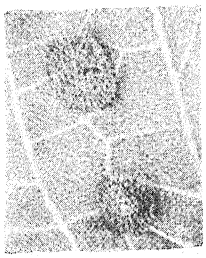
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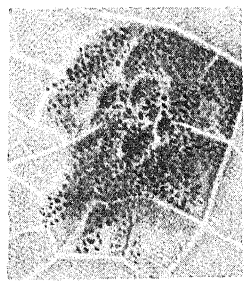
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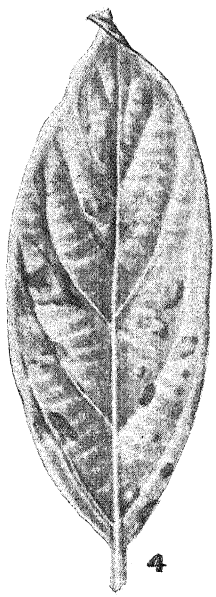
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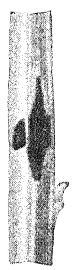
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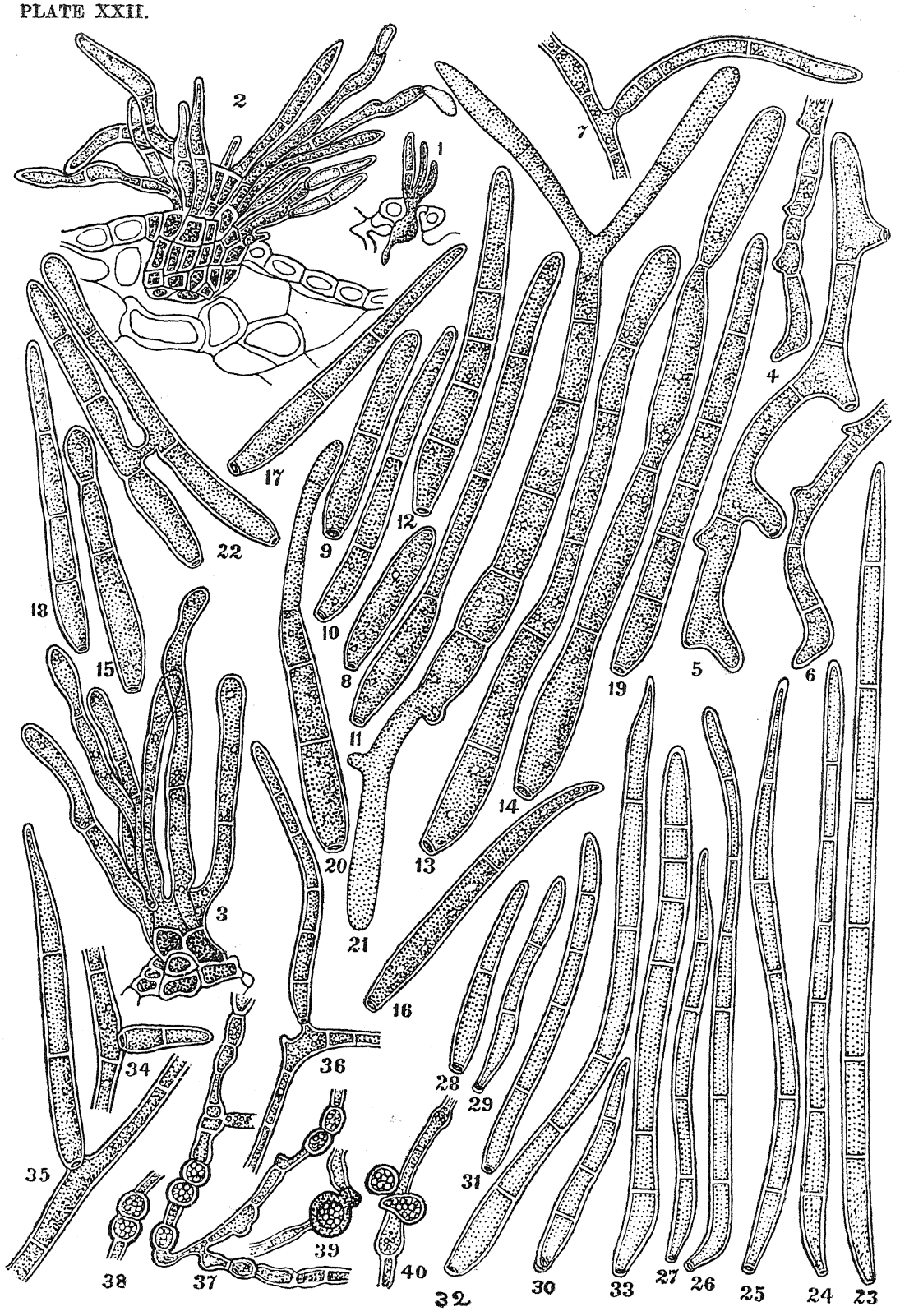
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(*For explanation see page 359.*)

PLATE XXII.



(For explanation see page 359.)

Conidia are hyaline to slightly greenish yellow in colour, multiseptate, abruptly obclavate sometimes vormiform, with indistinct scars less than 2μ in diameter at the distal end (Plate XXII, figs. 7-19). Constrictions near septa are very common (Plate XXII, figs. 8 and 9). In (CP) the size varies from 6·8μ-129·0μ ⩒ 3·4μ-5·1μ; septation 0-9, average being 38·7μ ⩒ 4·2μ; septation mode 2. In strain (CA), the size varies from 6·8μ-108μ ⩒ 3·4μ-5·1μ; septation 0-9, average being 36·8μ ⩒ 4·2μ; septation mode 2. From above it is clear that both strains are identical in their morphology. The cell contents of young conidia are perfectly hyaline but in mature spores it is light green and the contents are granular with number of oil globules. In tap water these germinate between 8-10 hours, at 30°C. (Plate XXII, figs. 20 and 22). All the cells of the multiseptate conidia do not lose viability when subjected to desiccation. In some conidia, some cells appear to be entirely empty and shrunken while in others the finely granular protoplasm is present. Conidia does not remain viable for a long period as no conidia germinate after about two months. Klotz [1923] found that conidia of *Cercospora apii* Fres. germinated after 170 days of drying; while Lehman [1928] observed that conidia from preserved specimen of *C. diazu* Maru germinated after 79 days. The conidia attained their maximum length under high humidity. The cells of the distal end of the conidia are longer than those at the proximal end. Conidia produced in culture are more hyaline than in nature.

III. Parasitism.

Both the strains (CA) and (CP) are unable to infect *Cajanus indicus* leaves when inoculated with mycelium alone. No infection takes place even when the mycelium is placed on nutrient drops containing 5 per cent. cane sugar *plus* 2 per cent. asparagin or on drops of stale filtrate in which the fungus was allowed to grow for 4, 8, 16 and 32 days.

When spore suspension is sprayed the incubation period is ten days in the case of mature leaves, and fifteen days in the case of immature leaves. The fungus was isolated from artificially infected leaves and single spore cultures obtained and when compared with the original, they proved to be identical. Infection of petioles and stems when inoculated also took infection.

Disinfected seeds were kept in potato-tubes containing sterilized Knops solution and when the seedlings were six days old, they were smeared with spore suspension and kept at 10°C., 20°C., 27·5°C., 30°C. and 35°C. Triplicate tubes were placed in each temperature. No infection took place below 20°C., and above 32·5°C., good infection at 20°C. and 25°C., moderate at 27·5°C. and slight at 30°C. and 32·5°C. Symptoms of the disease appeared after thirteen days and in the advanced stage leaves curled and became yellowish.

Infection takes place readily both in darkness and in light. The observations made agree with those of Klotz [1923] on *Cercospora apii*, Fres., but not with those of McKay and Pool [1918] on *Cercospora beticola* Sacc. on sugar beet who reported that infection probably took place only in the daytime.

The fungus when inoculated on *Dolichos Lablab*, *Glycine hispida*, Maxim, *Phaseolus acontifolius*, Jacq., *Phaseolus radiatus*, Linn., *Phaseolus mungo Linn. var. Roxburghii* and *Vigna catjang* Endl., failed to infect.

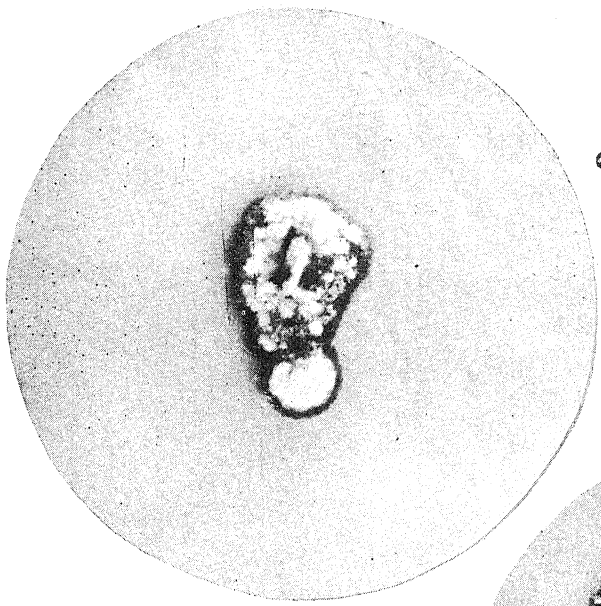
IV. Cultural studies of the fungus.

(A) Macroscopic characters.

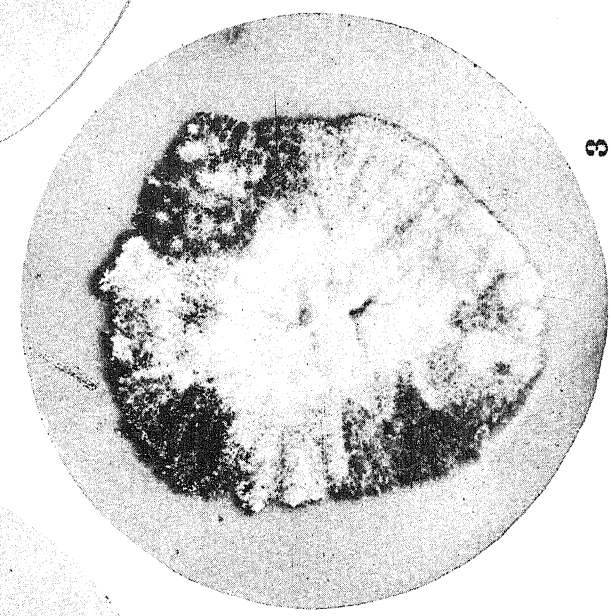
(*i*) *Growth on culture media.*—Both the strains when cultivated on a large number of culture media, showed remarkable differences in cultural characters. They differed from each other in (*a*) amount and colour of the aerial mycelium, (*b*) rate of linear growth, (*c*) colour and nature of the submerged mycelium, (*d*) colour of the substratum. A comparative statement of the two strains is given in Table I.

Table I.

Media	Strain (CA)	Strain (CP)
Coons' agar	*Aerial mycelium* abundant, cottony, light lilacy white; edge light greyish indigo and woolly. *Submerged mycelium*, bluish black with dark brown chlamydospores. *Substratum* light titamouse blue; edge light stone colour.	*Aerial mycelium* sparse, cottony, light purplish tinted white with patches of bright greenish grey mycelium. *Submerged mycelium* highly tortuous, dark brown with numerous chlamydospores. *Substratum*, dark forget-me-not blue, edge light horizon blue.
Richards' solution agar	*Aerial mycelium*, profuse, woolly, light sky coloured white, at places light paynes grey. *Submerged mycelium*, highly tortuous greenish brown with abundant chlamydospores. *Substratum*, dark cypress green.	*Aerial mycelium*, scanty, woolly, dark slate grey, edge light mouse colour. *Submerged mycelium*, dark cypress green. *Substratum*, dark ivy green, edge cypress green.
Oatmeal agar	*Aerial mycelium*, copious, cottony, light purplish tinted white, at places light paynes grey. *Submerged mycelium*, bluish black with abundant dark brown chlamydospores. *Substratum*, centre dull greenish grey; edge light plata indigo.	*Aerial mycelium*, scanty, woolly, light paynes grey. *Submerged mycelium*, bluish black with plenty of greenish brown chlamydospores. *Substratum*, dark bluish black.



2



3



1

(*For explanation see page 360.*)

TABLE I.—*contd.*

Media	Strain (CA)	Strain (CP)
Browns' synthetic agar	*Aerial mycelium*, profuse, cottony, light lilacy white. Growth very poor. *Submerged mycelium*, compact and bluish black. *Substratum*, greenish blue.	*Aerial mycelium*, very sparse cottony paynes grey. *Submerged mycelium*, light bluish green with sparse chlamydospores. *Substratum*, light cobalt blue.
Prune juice agar . .	*Aerial mycelium*, abundant loose, cottony, greyish white. *Submerged mycelium*, greenish grey with sparse chlamydospores. *Substratum*, light greyish indigo.	*Aerial mycelium*, moderate, woolly, light grey green. *Submerged mycelium*, dark brown, highly tortuous with abundant chlamydospores. *Substratum*, light blue carbonate of copper.
Beyrincks' agar . .	*Aerial mycelium*, sparse cottony, light purplish tinted white, spreading growth. *Submerged mycelium*, light greenish grey with no chlamydospores. *Substratum*, light fleshy white.	*Aerial mycelium*, absent. Growth tree-like branching profusely. *Submerged mycelium*, dark listre green. *Substratum*, light golden bronze green.
Cajanus indicus stem .	*Aerial mycelium*, abundant cottony, light purplish tinted white. *Sclerotial bodies*, abundant.	*Aerial mycelium*, less abundant than (CA), cottony, light mouse colour. *Sclerotial bodies*, abundant.
Wheat straw . .	*Aerial mycelium*, scanty cottony, light lilacy white. *Sclerotial bodies*, abundant.	*Aerial mycelium*, scanty, grey. *Sclerotial bodies*, abundant.

Both the strains were also grown on Doxs' agar, Hopkins' agar, Brown's starch medium, *rahar* leaf decoction agar, and plain agar and showed marked differences on cultural characters.

(*ii*) *Influence of depth of media.*—The effect of depth on medium upon linear rate of growth of the two strains was made on Coons' agar and Oatmeal agar at 27·5°C. and the results obtained were similar to those of *Cercospora dolichi* [Singh, 1933], *i.e.*, there is increase in the linear rate of growth with increase in the amount of medium. Similar results were obtained by Mitra [1931] on *Helminthosporium* species.

(*iii*) *Light relations.*—Effect of alternate light and darkness, continuous light from 100 watt electric lamp and continuous darkness, on linear rate of growth of both the strains was carried out and the strains were also grown on liquid solution (Coons'). It was found that the rate of linear growth is greater in alternate light and darkness, less in continuous darkness and least in continuous light. The retarding effect of continuous darkness and continuous light becomes more evident with time. Similar results were obtained with *C. dolichi* E. and E. [Singh, 1933].

(*iv*) *Relative humidity.*—The effect of different relative humidity on growth of these two strains was carried out and the method followed was similar as in *C. dolichi* by Singh [1933] and the results are given below in Table II.

TABLE II.

Relative humidity	Strains	15 days	25 days	35 days
47	(CA)	9·6 mm.	11·6 mm.	12·5 mm.
	(CP)	5·3 ,,	6·0 ,,	8·4 ,,
68	(CA)	12·6 mm.	14·9 mm.	20·6 mm.
	(CP)	10·5 ,,	10·6 ,,	19·0 ,,
70·4	(CA)	13·0 mm.	15·6 mm.	22·0 mm.
	(CP)	11·0 ,,	11·5 ,,	20·0 ,,
78·7	(CA)	13·2 mm.	16·0 mm.	27·2 mm.
	(CP)	11·3 ,,	12·0 ,,	24·4 ,,
92·3	(CA)	13·5 mm.	20·5 mm.	31·2 mm.
	(CP)	11·3 ,,	12·3 ,,	26·4 ,,
100	(CA)	13·6 mm.	20·8 mm.	32·9 mm.
	(CP)	11·5 ,,	15·0 ,,	29·3 ,,

From the above table, it will be seen that the optimum relative humidity for growth of both the strains (CA) and (CP) is 100 per cent. and that the fungus tolerates a wide range of relative humidity from 47-100.

(*v*) *Temperature relationships.*—The temperature relationships study of the two strains was made on Coons' agar, Richards' solution agar and Prune juice agar. No growth occurred at 5·5°C. and 37·5° in all media. On Richards' solution agar growth took place even at 37·5°C. Both the strains showed optimum growth at 27·5°C. in all media tried.

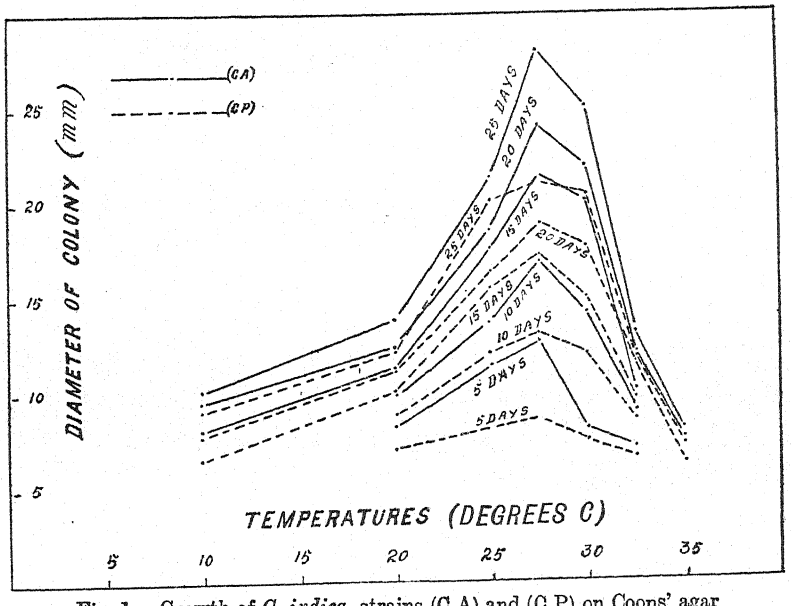


Fig. 1. Growth of *C. indica*, strains (C A) and (C P) on Coons' agar at various temperatures.

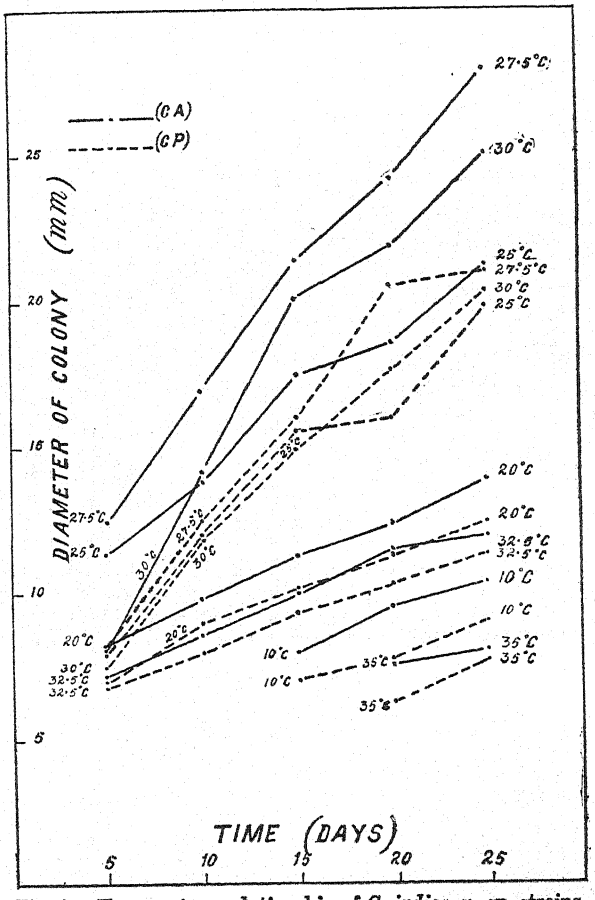


Fig. 2. Temperature relationship of *C. indica*, n. sp. strains (C A) and (C P) on Coons' agar.

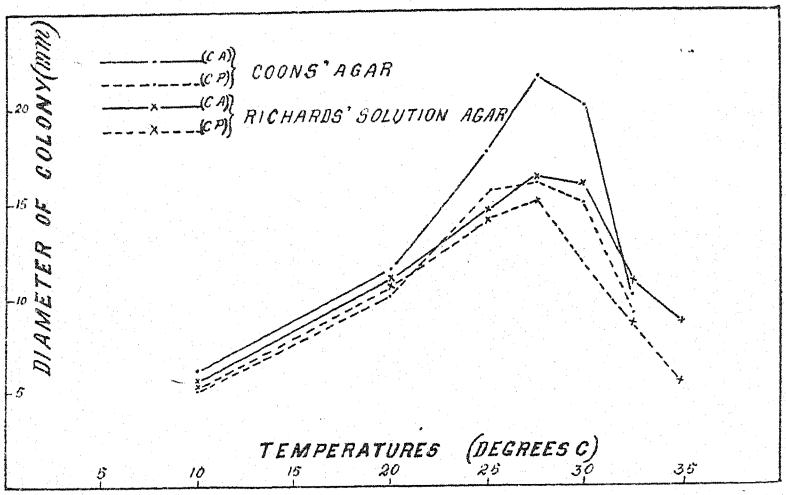


Fig. 3. Fifteen days' growth of *C. indica*, n. sp., strains (C A) and (C P) at various temperatures on Coons' agar and Richards'solution agar.

Figs. 1 and 3 illustrate (*a*) the relation between growth rate and temperature during a fixed time period and (*b*) the relation between growth rate and time at certain temperatures. From Figs. 1, 2 and 3 it is seen that the fungus grows well at temperatures from 20°C.—30°C. Both the strains show best growth between ten and fifteen days. After fifteen days growth slows down. The rate of linear growth of strain (CA) is greater than (CP) under identical conditions of growth.

(*vi*) *Concentrations.*—Both the strains were grown on different concentrations of Coons' solution. Dry weight of the mycelium was determined after a month's growth. The dry weight of the mycelium in both cases decreases either by increasing or by decreasing the concentration of the normal solution, and thus the best growth takes place at normal concentration of the medium. Table III shows the relative dry weight of both the strains.

TABLE III.

Strains	10 *N*	5 *N*	2 *N*	*N*	*N*/2	*N*/5	*N*/10	*N*/20	*N*/50	*N*/100
	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.	grm.
(CA)	·0246	·0267	·0277	·0345	·0134	·0103	·0015	·0014	·0031	·0009
(CP)	·0179	·0190	·0210	·0220	·0178	·0167	·0149	·0144	·0140	·009

The size of the floating colonies their number and intensity of colour reduce with the lowering of the concentrations. This reduction in the number of floating colonies also takes place with the increase in concentration, at 10 *N* only one big floating colony is present. From *N*/5—*N*/100 submerged portion of floating colonies is not as usual bluish green but pure white. At concentrations above normal, *i.e.*, 10 *N*, 5 *N*, 2 *N*, as well as in *N*, the dry weight of the mycelium of strain (CP) is less than (CA), while at concentrations below normal, *i.e.*, *N*/2, *N*/5, *N*/10, *N*/20, *N*/50 and *N*/100 reverse is the case.

(*vii*) *Importance of different constituents of Coons' solution.*—In order to determine the importance of different constituents of a synthetic solution on growth of (CA) and (CP) flasks of Coons' solution containing 50 c. c. of the medium with one constituent left out were inoculated and kept at 31°C. and dry weight of the mycelium determined after sixty-one days and are given in Table IV.

TABLE IV.

Strains	AVERAGE DRY WEIGHT OF THE MYCELIUM				
	Normal	No $MgSO_4$	No asparagin	No KH_2PO_4	No maltose
(CA)	·0373 grm.	·0200 grm.	·0097 grm.	·0300 grm.	·0002 grm.
(CP)	·0538 ,,	·0205 ,,	·0048 ,,	·0508 ,,	·0007 ,,

From the above table it will be seen that the constituents of Coons' solution in order of their importance can be arranged in the following order, *viz.*, maltose, asparagin, magnesium sulphate and potassium acid phosphate. The importance of maltose is greatest since in cultures with no maltose the growth is very poor and the colonies are white in colour. Acid phosphate is of least importance. Except in Coons' solution with no asparagin, the growth of strain (CP) is greater than (CA).

(B) MICROSCOPIC CHARACTERS.

(*i*) *Characteristic of mycelium.*

Aerial mycelium of (CA) is at first hyaline, straight with septa at long intervals, while that of (CP) is usually smoky brown; in older cultures it becomes slightly

yellowish and highly tortuous with abundant chlamydospores in the case of (CA) while in (CP) it becomes more highly torulated with abundant chlamydospores than in strain (CA).

Submerged mycelium of (CP) is thicker than the aerial mycelium and greenish grey with septa at short intervals. In older cultures it becomes dark brown, highly tortuous and torulated. Chlamydospores are found in all media tried except plain agar. Due to the abundance of chlamydospores the submerged mycelium of (CP) is much more highly tortuous than that of (CA) and present even on plain agar.

(ii) Size and septation of spores as influenced by different factors.

(a) Cultural medium.—To see the effect of different media on size and septation of spores both the strains were cultivated on a number of media and the range of length, width and septation of spores were determined at 25°C. on Coons' agar, Prune juice agar, *Cajanus indicus* stems, *C. indicus* leaf decoction agar and wheat straw. Very elongated and thin conidia were produced on *Cajanus indicus* stem and *Cajanus indicus* leaf decoction the proximal end of the spores is small and narrow while the distal end is thin and long (Plate XXII, figs. 23-27). The intensity of sporulation of the two strains is given in Table V.

TABLE V.

Strains	Coons' agar	*Cajanus indicus* leaf decoction agar	Wheat straw	Prune juice agar	*Cajanus indicus* stem
(CP)	XXX	XXXX	X	XXX	XX
(CA)	—	X	—	—	XX

— = no sporulation; X= slight; XX= good; XXX= very good; XXXX= best.

From Table V it will be noticed that strain (CA) does not form spores on Coons' agar, wheat straw and Prune juice agar. A comparison of spore measurement of both the strains on *Cajanus indicus* leaf decoction agar and stem shows some difference in spore length, though they are alike in shape of the spore, width and septation. The size and the septation of spores of strain (CP) is greatest on Prune juice agar and least on Coons' agar while on these medium the strain (CA) does not sporulate.

TABLE VI.

Strains	Media	Length (μ)				Width (μ)		Septation		Septation mode
		Range	Average	S. D.	C. V.	Range	Average	Range	Average	
(CP)	Coon's agar	23·4-136	61·1±1·5	23·58	37	1·7-3·4	3·2	1-10	4	3
(CP)	Wheat straw	20·4-187	70·3±1·1	15·0	21·3	1·7-3·4	3·2	1-12	5	4
(CP)	Prune juice agar.	10·2-278·8	73·4± ·9	13·8	10·8	1·7-3·4	3·3	0-16	6·3	6
(CP)	*Cajanus indicus* stem.	13·6-170	64 ± ·67	10·0	15·6	3·4-5·1	3·2	0-11	4·8	5
(CA)		10·2-183·6	67 ± ·8	12·0	17·9	3·4-5·1	3·2	0-11	4·8	5
(CP)	*Cajanus indicus* leaf decoction agar	12 -148·6	68·5±1·1	15·0	21·8	1·7-3·4	3·4	1-11	5·4	4
(CA)		17·0-156·4	72·5±1·1	17·0	23·2	1·7-3·4	3·5	0-13	5·6	4

(*b*) *Temperature.*—The effect of temperature on size and septation of spores was determined only for strain (CP) on Coons' agar and the data are given in Table VII. Twenty-five days old culture was used in each case (Plate XXII, figs. 28-36).

TABLE VII.

Temperatures °C.	Length (μ)				Width (μ)		Septation		Septation mode
	Range	Average	S. D.	C. V.	Range	Average	Range	Average	
10	No sporulation								
20	20·4-173·4	61·8±1·8	28	41	3·4-5·1	3·5	1-12	4	4
25	23·4-136	61·15±1·5	23·5	37	2·5-3·4	3·4	1-10	4	3
27·5	20·4-129·2	58·65±1·4	20·9	35	2·5-3·4	3·4	1-8	3·9	4
30	20·4-102·8	54·7± ·9	13·8	20	2·5-3·4	3·4	1-7	4	3
32·5	6·8-78·2	36·4±1·2	17·7	48	2·5-3·4	3·2	1-5	2·4	1
35	6·8-68	32·8±1·8	19·0	59	1·7-3·4	2·1	0-3	1·3	1

From Table VII it will be seen that the size and septation of spores is greatest between 20 and 25°C. At 32·5°C., 35°C., the spores formed are smaller in size and with few septa. Sporulation is best at 20°C.

(iii) Sclerotial formation.

Sclerotia like bodies are formed in very old cultures. They are abundantly formed on wheat straw and *Cajanus indicus* stem. These are formed by the irregular divisions of the cells of the mycelium. Often these are more or less rounded bodies of dark brown colour.

(iv) Chlamydospores.

These are found at all temperatures. In strain (CP) these are dark brown with thick walls while in strain (CA) they are greenish brown and thin walled rounded bodies which give the mycelium a beaded appearance (Plate XXII, figs. 37-40).

V. Hydrogen-ion concentration.

Modified Richards' solution of Karrer and Webb [1920] was adjusted to various hydrogen-ion concentrations and four flasks were prepared for each pH value. One of these was put as control while the other three were inoculated. All the flasks were incubated at 27·5°C. for 79 days. After that period the pH values of the filtrate as well as of the control together with the dry weight of the mycelium were determined.

Experimental results and conclusion.—The data are presented in Table VIII and the growth is represented graphically in Fig. 4. Dry weight of the mycelium represents the average dry weight of the mycelia from the three flasks. Growth starts abruptly between pH 2·5 and 2·9, there being no growth at pH 1·7, 2·1 and 2·5, while at pH 2·9 there is fair amount of growth. The growth is uniform over a range of pH 2·9—7·1. Maximum growth occurs at pH 6·7. After that there is a sudden fall up to pH 7·1, beyond which no growth takes place. On the acid side of neutrality no growth took place at pH 1·7, 2·1 and 2·5, while on the alkaline side of neutrality no growth at pH 7·7 and beyond was observed. Only one maximum is obtained and that is at pH 6·7 on the acid side of neutrality. Thus pH 1·7, 2·1, 2·5, 7·7, 8·1 and 9·1 should be regarded as toxic hydrogen and hydroxyl ions, for no growth of the fungus occurred at these pH values.

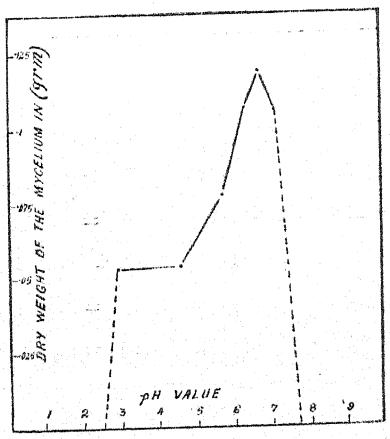


Fig. 4. Dry weight of the mycelium of *C. indica*, strains (C P) in 79 days at 27·5°C. at various hydrogen-ion concentrations.

TABLE VIII.

The growth of (CP) and the changes in reaction induced by growth in modified Richards' solution at different hydrogen-ion concentrations at 27·5°C. in darkness after 79 days.

H-ion concentration			Average dry weight of the mycelium
Initial	Final		
	Control	Inoculated	
1·7	1·7	1·7	No growth
2·1	2·1	2·1	,, ,,
2·5	2·5	2·5	,, ,,
2·9	2·9	2·9	·0556 grm.
4·6	4·1	2·9	·0558 ,,
5·7	5·3	2·9	·0785 ,,
6·3	6·0	2·9	·1100 ,,
6·7	6·2	2·9	·1145 ,,
7·1	6·5	2·9	·1031 ,,
7·7	7·3	7·3	No growth
8·1	7·8	7·8	,, ,,
9·1	8·8	8·8	,, ,,

The fungus during its growth on the modified Richards' solution produced marked changes in the reaction of the medium. The changes in pH value that the control undergoes during the period are shown in Table VIII. It is seen from the table that there has been very little change in pH values of the controls on the acid side of neutrality, while in those on the alkaline side there is remarkable shift in pH values. The pH 1·7, 2·1 and 2·5 remained constant after 79 days, while pH 7·1, 7·7, 8·1 and 9·1 shifted to pH 6·5, 7·3, 7·8 and 8·8 respectively. The pH 2·9, 4·6, 5·7, 6·3, 6·7, 7·1 of the inoculated flasks all shifted to a constant pH 2·9, which therefore represents the acidity of the medium on which the fungus grew well. Similar results were obtained on *C. dolichi* by Singh [1933]. In certain vegetable decoctions the change has been reported to be on the alkaline side of neutrality.

The question as to how the fungus passes its dormant period on the surface of Pusa soil which has got a pH value as high as 8·2 while the fungus can not grow beyond pH 7·1 could not be accounted for in view of the fact that the limiting hydrogen-ion concentration is the same in the soil as in the culture solution. But as so many chemical reactions are constantly going on in the soil, it is no wonder that the dormant mycelium could be able to save itself from being totally destroyed and with the return of favourable conditions gives rise to a new crop of conidia.

A study of the growth of the fungus on modified Richards' solution of pH 4·5 was carried out, and the records in the shift of pH of the inoculated and the control were made once after each fifteen days' interval up to sixty days, as given in Table IX below.

TABLE IX.

Days	pH control	Average pH of inoculated	Average dry weight of the mycelium
15	4·5	3·8	·0472 grm.
30	4·3	3·3	·0650 „
45	4·3	2·9	·0739 „
60	4·3	2·9	·0884 „

From Table IX it is seen that there is no change in pH value of the control after 15 days, but the pH of the inoculated is changed from pH 4·5 to 3·8. In 60 days the acidity of the control is increased from pH 4·5 to 4·3, while that of the inoculated ones from 4·5 to 2·9. pH remains constant between 45 and 60 days. The greatest increase in the dry weight of the mycelium takes place between fifteen and thirty days.

VI. Diagnosis of the new species.

There are two species of Cercospora known on *Cajanus indicus*, *viz.*, *Cercospora cajani* (P. Henn) and *Cercospora instabilis* Rangel. Rangel has changed *Cercospora cajani* (Henn) into *Vellosiella cajani* (P. Henn) Rangel. The two strains of *Cercospora* under study, one isolated from Allahabad (CA) and the other from Pusa (CP) are, however, quite different to the two species mentioned above. A comparison of the two strains with the other known species is given below in Table X.

TABLE X.

	Vellosiella cajani (P. Henn) Rangel or *Cercospora cajani* (Henn)	*Cercospora instabilis* (Rangel)	*Cercospora* sp. from Allahabad and Pusa (CA) and (CP)
Symptoms . .	Round spots of a chestnut brown colour with dark brown margin from 2-3 cm. in diameter, on both sides of the leaves. Spots irregular, scattered or sometimes grouped together tufted on the upper surface.	Small angular spots of a dark brown colour with a dark red margin on both sides of the leaves, on branches and fruits also. Spots scattered and aggregated and tufted on both surfaces.	Small irregular spots of a dark brown colour with light brown margin on undersurface of leaf, but in severe cases on both sides. It first appears on the undersurface of the leaves. Spots usually separate, but often several coalesce forming large diseased areas as large as 15 mm. × 5 mm. These spots are tufted on the undersurface of the leaves. Infection of petiole and stem also occurs but never of fruits.
Conidiophores . .	Arising through stoma in clusters, tips bent, septate, pale brown, cylindrical 4-6μ in diameter.	Mostly erect or twisted, abruptly bent like knee joint, septate, of sooty colour, stromata minute protruding 50-80μ ⩒ 4-6μ mostly erect or curved. Stromata 20-30μ ⩒ 1·5-3μ.	Branched, branching alternate mostly bent arising through stoma in clusters. Stromata very prominent found in the air spaces and sometimes though rarely protruding outside the leaf. Light brown while young dark brown when mature with prominent giniculations on all sides. 28·0μ—168·0μ ⩒ 3·4—7·0μ. Septa 2-13.
Conidia . . .	Apically attached bud shaped sometimes forming a part of the conidiophore oblong, sometimes obtuse straight or slightly curved. One septate rarely 2-3 septate, non-constricted, sub-hyaline to pale brown in colour 20-30μ ⩒ 4·6μ media (20-24μ ⩒ 5-6μ).	Club shaped or vormiform, multiseptate, bent hyaline. 80-200μ ⩒ 2·5-4μ.	Hyaline or slightly greenish yellow multiseptate, abruptly obclavate sometimes vormiform with indistinct scars less than 2μ in diameter. Constricted near septa. Strain (CP) (6·8μ—129μ) ⩒ (3·4μ—5·1μ). Septation 0-9. Average (38·7μ ⩒ 4·2μ). Septation mode 2. Strain (CA) (6·8μ—108μ) ⩒ (3·4μ—5·1μ). Septation 0-9. Average 36·8μ ⩒ 4·2μ. Septation mode 2.

From the above table it is seen that both the Indian strains are quite different to those previously known and recorded on *Cajanus indicus*. The Indian strains of *Cercospora* further do not agree with any of the *Cercospora* known on any other pulse. Therefore the author considers it to be a new species consisting of two strains (CA) and (CP) which differ from each other only in cultural characters which are of minor importance as regards determination of species as previously mentioned in the text and the name *Cercospora indica* is proposed.

The diagnosis is as follows :—

Cercospora indica n. sp., spots minute, irregular, 1-2 mm. in diameter, scattered or sometimes aggregated, dark brown with light brown margin, tufted mostly on the under surface, in advanced cases on both surfaces. Petiole and stem are also infected. Conidiophores, branched, branching alternate, mostly bent arising from stoma in clusters, light brown to dark brown with prominent giniculations, 28μ-168μ ⩒ 3·4μ-7μ. in diameter, 2-13 septa. Stromata prominent in air spaces rarely protruding out of the stoma. Conidia, hyaline, or slightly greenish yellow, multiseptate, abruptly obclavate sometimes vermiform with indistinct scars less than 2μ in diameter, often constricted near septa. 6·8μ-129μ⩒3μ-5μ in diameter, average by 37μ ⩒ 4μ. Septation 0-9 with mode at 2.

Habitat.—On leaves, petioles and stems of *Cajanus indicus* Spreng. Allahabad and Pusa.

Type specimen.—Deposited in Pusa herbarium and Allahabad University herbarium.

VII. Summary.

1. The leaf-spot disease of *Cajanus indicus* caused by two strains of *Cercospora* is of very common occurrence. The symptoms of the disease and the morphology of the fungus are described.

2. Artificial infection takes place through spores only. Infection of other pulses does not take place. Between 20°C. and 25°C. infection occurs readily.

3. A comparison of cultural character of the two strains on a number of media shows remarkable differences.

4. Growth of strain (CA) is greater than (CP) in all media. Using Coons' agar, it was found in case of both the strains that growth of the fungus increases with the increase in the amount of media; it is more in alternate light and darkness, less in continuous darkness and least in continuous light. It grows through a wide range of relative humidity from 47-100 per cent. Best growth of both strains takes place at 100 per cent. humidity.

5. Optimum temperature for growth for both the strains is 27·5°C. No growth at 5·5°C. and 37·5°C. except on Richards' solution agar where the growth also takes place at 37·5°C.

6. Growth of both the strains is retarded, both by diluting and concentrating Coons' solution. Maltose is the most important constituent of Coons' solution for growth of both the strains, asparagin, magnesium sulphate, potassium acid phosphate being of less importance.

7. Best sporulation takes place at 20°C.-25°C. No formation of spores at 10°C. or below. Length and septation of spores is greatest at 20°C.-25°C., and decreases with the increase or decrease of temperature.

8. The fungus renders the medium on which it grows acid, and tolerates a wide range of pH 2·9-7·1. Optimum growth is at pH 6·7. No growth at pH 1·7, 2·1, 2·5, 7·7, 8·1 and 9·1 has been observed.

9. Both the strains have been found to belong to one species which has hitherto not been described and is named *C. indica* the diagnosis of which is given.

ACKNOWLEDGMENTS.

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IX. Explanation of Plates.

PLATE XXI.

(The figures are reduced to 2/3.)

Figs. 1 and 2.—Early stages in the infection of leaf. Undersurface. (×1½).
Fig. 3.—Later stage of the same. (×1½).
Fig. 4.—Advanced stage in the infection of leaf. Upper surface. (×1½).
Figs. 5 and 6.—Enlarged young and old spots of leaf. (×8).
Fig. 7.—A portion of petiole showing infection spots. (×1½).
Fig. 8.—A portion of stem showing infection spot. (×1½).

PLATE XXII.

(The figures are reduced to 2/3.)

Fig. 1.—A portion of T. S. of leaf showing emergence of conidiophores through a stoma. (×800).
Fig. 2.—A portion of T. S. of leaf showing a fascile of conidiophores bearing conidia. (×800).

Fig. 3.—A fascile of conidiophores from host. (×800).

Figs. 4, 5 and 6.—Conidiophores from host. (×1,850).

Figs. 7, 8, 9, 10, 11, 12, 13, 14 and 15.—Typical conidia of Strain (CP) from host. (×1,850).

Figs. 16, 17, 18 and 19.—Typical conidia of the strain (CA) from host. (×1,850)

Figs. 20 and 21.—Germinating conidia from host. (×1,850).

Fig. 22.—Two conidia fusing from host. (×1,850).

Figs. 23 and 26.—Typical conidia from sterilized *rahar* stem at 20°C., 25 days old culture. (×1,850).

Figs. 24, 25 and 27.—Typical conidia from *rahar* leaf decoction agar at 30°C., twenty-five days old culture. (×1,850).

Figs. 28, 29, 30, 31, 32 and 33.—Typical conidia from Coons' agar at 27·5°C., 25 days old culture. (×1,850).

Figs. 34 and 35.—Conidiophores with conidia attached from Coons' agar at 32·5°C., one month old cultures. (×1,850).

Fig. 36.—Conidiophore with a conidia attached on oat meal agar at 30°C., one month old cultures. (×1,850).

Figs. 37 to 40.—Stages in the formation of chlamydosphores, from Coons' agar at 27·5°C. (×800).

PLATE XXIII.

Fig. 1.—Twenty-five days old culture of *C. indica*, strain (CA) on Coons' agar at 27·5°C.

Fig. 2.—Twenty-five days old culture of *C. indica*, strain (CP) on Coons' agar at 27·5°C.

Fig. 3.—107 days old culture of *C. indica*, strain (CA) on rice meal agar at 27·5°C. showing false sectors.

STATISTICAL NOTES FOR AGRICULTURAL WORKERS.

NO. 15. ANALYSIS OF ROTATIONAL EXPERIMENTS WITH COTTON, GROUNDNUT, AND *JUAR* IN BERAR, WITH NOTES ON DESIGNS FOR ROTATIONAL EXPERIMENTS.

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(With two text-figures)

INTRODUCTION.

Messrs. D. N. Mahta and D. L. Janoria have described in their paper* on "Groundnut as a Rotation Crop with Cotton in Berar", the important experiments conducted by them during the eight years 1923-31.

The main object of their experiments was to investigate the most economic rotation of crops for cotton in Berar, with groundnut and *juar* as possible subsidiary crops. In order to attain the maximum economic return it is, therefore, necessary to take into consideration the prices as well as the yields of the different crops.

Our object is to make the expected return in money as large as possible (on the average taken over a number of years). The enquiry, therefore, naturally splits up into two portions—one primarily agricultural concerned with the yields, and the other primarily economic concerned with the prices and the cost of cultivation. In this note I have considered only the agricultural aspect of the question.

THE EFFECT OF ROTATION ON ACTUAL YIELDS.

All the different rotations were conducted on separate fields. This has rendered an adequate statistical analysis impossible for the reasons explained below. The factors producing differences in yield in the present experiment may be broadly grouped under four heads :—

(1) Effect of different rotations.
(2) Soil-differences in different fields or different parts of the same field.
(3) Effect of different seasons.
(4) Random fluctuations.

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* *Ind. J. Agric. Sci.* Vol. III, Part V, Oct. 1933, pp. 917-32.

The object of the present experiment is to study the effect of different rotations on the yield. In order to reach a valid estimate of this effect it is necessary to eliminate the effects due to the other factors.

It is not possible to estimate or eliminate satisfactorily the effect of intrinsic differences in soil-fertility in the absence of random replications of different rotations in the same field. This constitutes an inherent defect of the original design of the present experiment and cannot be got over by any statistical expedient.

If, however, we ignore these intrinsic soil-differences, that is, assume that the average fertility of all the fields were originally more or less equal, we get the following results.

The actual mean yield of cotton (in lbs. per plot of 1/10th acre) for the different rotations are shown in Table I. The standard error of each mean is given in the last column.

TABLE I.

Mean yield of cotton (in lbs. per plot of 1/10th acre).

Serial number	Rotation	Total number of		Mean yield	Standard error of mean
		Years	Plots		
1	Cotton, groundnut	4	20	64·60	7·295
2	Cotton, *juar*, groundnut . . .	3	15	73·67	4·391
3	Cotton, cotton, *juar*, groundnut . .	4	18	68·52	9·182
4	Cotton, cotton, *juar*	4	20	47·10	4·855
5	Cotton, *juar*	4	20	37·55	1·705
6	Cotton every year (control A) . .	8	40	32·75	2·222
7	Cotton, cotton, groundnut . . .	4	20	54·10	5·349
8	Cotton every year (control B) . .	6	30	37·97	2·715

Rotations 2, 3 and 1 give the highest yields of cotton and do not differ significantly among themselves. Rotations 7 and 4 come next and differ appreciably from Rotation 2 but not from Rotations 3 and 1. Rotations 8, 5 and 6 which give the lowest yields do not differ appreciably among themselves, but differ significantly from Rotations 2, 3, 1 and 7, while Rotation 6 alone differs from Rotation 4.

The mean yields of groundnut for different rotations (with standard errors) are shown in Table II. It will be noticed that Rotation 7 gives an appreciably

higher yield than Rotations 1 and 2, and Rotation 3 an appreciably higher yield than Rotation 2, while other differences are statistically insignificant.

TABLE II.

Mean yield of groundnut (pods) (in lbs. per plot of 1/10th acre).

	Rotation	Total number of		Mean yield	Standard error of mean
		Years	Plots		
1	Cotton, groundnut	4	18	156·55	11·87
2	Cotton, *juar*, groundnut . . .	2	8	139·40	10·82
3	Cotton, cotton, *juar*, groundnut . .	1	5	195·00	12·14
7	Cotton, cotton, groundnut . . .	2	10	202·30	11·78
	General mean .	..	41	172·78	7·226

The mean yeld of *juar* for different rotations (with standard errors) are shown in Table III. None of the differences are significant, and we must conclude that the mean yield of *juar* is not appreciably influenced by the crop rotations used.

TABLE III.

Mean yield of juar (*green*) (*in lbs. per plot of 1/10th acre*).

	Rotation	Total number of		Mean yield	Standard error of mean
		Years	Plots		
2	Cotton, *juar*, groundnut . . .	2	10	143·7	9·38
3	Cotton, cotton, *juar*, groundnut . .	1	5	168·0	11·50
4	Cotton, cotton, *juar*	2	10	144·3	6·23
5	Cotton, *juar*	3	15	156·8	9·88
	General mean .	..	40	151·80	4·895

From the above analysis it is clear that for certain rotations the differences in the yield of different crops are inappreciable; for example between 1 and 2, 1 and 3, 1 and 4; 2 and 3; 3 and 4; 3 and 7; 4 and 5, 4 and 7, 4 and 8; 5 and 6, 5 and 8; 6 and 8. In these cases, so far as the yields of crops are concerned, rotational differences cannot be considered as established.

Seasonal effect.

In the above discussion we have so far neglected the effect of different seasons. This, however, is by no means negligible as can be easily seen from Table IV which gives the mean yield of cotton for different rotations in different years. Standard errors are also given in each case.

TABLE IV.

Mean yield of cotton (in lbs. per plot of 1/10th acre)

Year	1923-24	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31
1 Cotton, groundnut .	31·8±2·56	G	50·6±6·31	G	75·8±10·99	G	100·2±13·40	G
2 Cotton, *juar*, groundnut.	..	55·4±4·50	*J*	G	90·0±2·85	*J*	G	75·6±4·80
3 Cotton, cotton, *juar*, groundnut	..	..	43·8±3·46	32·3±(3)2·54	*J*	G	122·0±8·44	61·4±4·06
4 Cotton, cotton, *juar* .	..	..	33·6±1·17	37·2±5·50	*J*	37·0±3·15	80·6±5·49	*J*
5 Cotton, *juar* . .	..	37·4±2·84	*J*	39·0±5·50	*J*	42·0±1·22	*J*	31·8±1·32
6 Cotton every year (control A)	23·8±3·47	25·4±3·23	19·0±3·46	22·8±2·76	55·4±4·08	25·2±1·91	43·2±4·67	41·8±2·40
7 Cotton, cotton, groundnut	..	..	41·0±9·90	42·2±12·40	G	90·2±3·35	43·0±16·41	G
8 Cotton every year (control B)	..	..	38·0±3·27	46·6±4·38	77·2±2·97	36·2±1·16	44·2±3·89	46·6±1·50
9 General standard error of a single mean	3·05	3·59	3·99	4·69	6·21	1·93	7·87	3·14

TABLE V.

Seasonal effects (actual yields of cotton)

Rotation	Between years		Within years		Total		Ratio of variances	
	D. F.	Variance	D. F.	Variance	D. F.	Variance	Observed	5 per cent.
1	3	44,41·07	16	4,31·23	19	10,64·36	10·3	3·24
2	2	15,43·80	12	80·14	14	2,89·24	1,92·6	3·89
3	3	71,79·60	14	1,47·05	17	13,87·56	48·8	3·34
4	3	25,40·87	16	83·33	19	4,71·36	30·5	3·24
5	3	85·67	16	51·88	19	58·16	1·65	3·24
6	7	8,42·63	32	56·26	39	1,97·40	14·9	2·39
7	3	28,99·33	16	1,36·0	19	5,72·32	21·3	3·24
8	5	10,52·84	24	52·03	29	2,21·14	20·2	2·62

For any particular rotation, it will be noticed that the yields in different seasons differ significantly. This is brought out still more clearly in Table V, in which the variances due to seasons are shown for each rotation.

It will be noticed that the fluctuations due to the differences in the meteorological conditions in different seasons were very large (often of the order of 20 times or more of the residual variance), and statistically significant in the case of all rotations except No. 5.

The yield of groundnut in the different seasons are shown in Table VI, and the corresponding analysis of variance in Table VII.

TABLE VI.

Mean yield of groundnut

Year	1	2	3	7
	Cotton, groundnut	Cotton, *juar*, groundnut	Cotton, cotton, *juar*, groundnut	Cotton, cotton, groundnut
1924-25	1,59·0	C	—	—
1925-26	C	*J*	C	C
1926-27	99·7	1,19·0	C	C
1927-28	C	C	*J*	2,34·0
1928-29	2,30·0	*J*	1,95·0	C
1929-30	C	1,59·8	C	C
1930-31	1,37·2	C	*J*	1,70 6

TABLE VII.

Analysis of variance : Groundnut

Rotation	YEARS		PLOTS		RESIDUAL		TOTAL		RATIO OF VARIANCE	
	D. F.	Variance	D. F.	Variance	D. F.	Variance	D. F.	Variance	Years	Plots
1	3	12,519·33	4	200·75	10	467·80	17	2,531·71	26·76	<1·0
2	1	3,121·00	4	570·50	2	1,146·00	7	936·28	2·72	<1·0
3	—	..	4	737·00	—	—	4	737·00	—	—
7	1	9,581·00	4	513·25	4	213·50	9	1,388·67	44·93	2·40

The seasonal effect is definitely significant in the case of Rotations 1 and 7, inappreciable in the case of Rotation 2, and indeterminate in Rotation 3.

The mean yield of *juar* is given in Table VIII. From a glance at the critical differences given at the bottom it is seen that the seasonal effect is inappreciable.

TABLE VIII.

Mean yield of juar.

Year	2	3	4	5
	Cotton, *juar*, groundnut	Cotton, cotton, *juar*, groundnut	Cotton, cotton, *juar*	Cotton, *juar*
1924-25	C	—	—	C
1925-26	156·6	C	C	191·0
1926-27	C	C	C	C
1927-28	C	168·0	144·0	124·8
1928-29	130·8	C	C	C
1929-30	C	C	C	154·6
1930-31	C	C	144·6	C
S. E. of mean	13·26	—	8·81	17·12

In view of the large magnitude of the seasonal effect for cotton and groundnut, we may compare the yields for different rotations from year to year. But in doing so it is necessary to keep in mind the initial differences in soil fertility.

In each of the three basic years, 1923, 1924, and 1925, in which new experiments were started, all the fields were treated uniformly. Had there been no difference in soil fertility, the yields in the same basic year must, therefore, have been appreciably equal. In actual fact, the yields in different fields in the same basic year differed significantly as can be seen from Table IX. The standard error of a single mean (of 5 plots) for each year was calculated from the fluctuations within fields.

TABLE IX.

Mean yield of cotton in basic years.

Years	Rotation								Standard error
	1	2	3	4	5	6	7	8	
1923-24 .	31·8	..	..	..	..	23·6	..	..	3·05
1924-25 .	..	55·4	..	..	37·6	..	..	..	3·59
1925-26 .	..	..	43·8	33·6	..	..	41·0	58·0	3·99

In view of the statistically significant differences in yield for different fields in the same basic year, we must conclude that the intrinsic fertility of the different fields were appreciably different.

COMPARISON WITH CONTROL A (ROTATION 6).

It will be remembered that Rotation 6 (cotton every year) was used as the "control". The differences in the yield of cotton from Rotation 6 are given in Table X. The critical differences were taken as 3 times the corresponding standard errors which were calculated from the fluctuations within fields.

TABLE X.

Differences in yield of cotton from control A (Rotation 6).

Year	1	2	3	4	5	6	7	Critical difference (3 × S. E.) of single mean
	Cotton, groundnut	Cotton, *juar*, groundnut	Cotton, cotton, *juar*, groundnut	Cotton, cotton, *juar*	Cotton, *juar*	Cotton, cotton, groundnut	Cotton every year (control B)	
1923-24 .	+8·0	—	—	—	—	—	—	9·15
1924-25 .	G	+30·0	—	—	+12·0	—	—	10·77
1925-26 .	+31·6	*J*	+24·8	+14·6	*J*	+22·0	+19·0	11·97
1926-27 .	G	G	+9·5	+14·4	+16·2	+19·4	+23·8	14·07
1927-28 .	+20·4	+34·6	*J*	*J*	*J*	G	+21·8	18·63
1928-29 .	G	*J*	G	+11·8	+16·8	+65·0	+11·0	5·79
1929-30 .	+57·0	G	+78·8	+37·4	*J*	—0·2	+1·0	23·61
1930-31 .	G	+33·8	+19·6	*J*	—10·0	G	+4·8	9·42

Rotation 1 (Cotton and groundnut alternately). Although the difference in yield in the basic year (1923) is not significant on the 5 per cent. level, the difference (+8, which is over 33 per cent. of the control yield 23·8) is large enough to be suggestive. Ignoring soil differences, the differences in the yields of cotton were definitely significant in later years.

Rotation 2 (Cotton, *juar*, groundnut). The intrinsic difference (+30·0) in the basic year (1924) was very high being 118 per cent. above 'control' (25·4), and was definitely significant. The differences in later years were also all significant. The differences in yield, however, remained practically constant and the same as in the basic year. This naturally suggests that these differences were caused by the initial soil differences (which remained steady during the whole period), and that the effect of the rotation, if any, was completely masked by these overwhelming soil differences.

Rotation 3 (Cotton, cotton, *juar*, groundnut). The initial difference in yield in 1925 was significant, and must be ascribed to intrinsic soil differences. The difference in yield was inappreciable in 1926, but after a crop of *juar* and then a crop of groundnut there was a remarkable recovery in the yield in 1929 which was followed by a decreased yield in a second cotton year in 1930. In view of the

large fluctuations in the differences it is, however, difficult to draw any reliable conclusions.

Rotation 4 (Cotton, cotton, *juar*). The differences in yield were throughout significant, and were on the whole fairly steady except in 1929 when there was a much greater yield. In view of the initial soil differences it is, however, difficult to ascribe the gain in yield to any rotational effect.

Rotation 5 (Cotton and *juar* alternately). The differences were statistically significant in 1924, 1926 and 1928, but there was an actual decrease as compared to the 'control' in 1930. In view of the initial difference in yield, and the drop in 1930, it does not appear probable that Rotation 5 possesses any real advantage over Rotation 6.

Rotation 7 (Cotton, cotton, groundnut). In the first two seasons the differences were steady and significant. In 1928, following a groundnut crop, there was a very high yield; but in 1929, in a second cotton season, there was practically no advantage over control A (Rotation 6).

Rotation 8 (Cotton every year). Initially there was a big difference in yield in 1925 which was very definitely significant. The difference, however, decreased steadily in successive seasons, until in 1929 and 1930, Rotations 6 and 8 both had practically the same yield. This suggests a gradually increasing exhaustion of the soil in the field under Rotation 8. It is, however, curious that the first 'control' field A (Rotation 6) does not show any trace of such effect. This would appear to indicate that the 'control' field A under Rotation 6 was initially poor in fertility or was already exhausted, a view which is supported by the fact that this field gave consistently the lowest yields in all three basic years 1924, 1925 and 1926.

Comparison with control B (Rotation 8).

Rotation 8 also consists of cotton every year, and there is no reason why it should not be used as a 'control'. We have already seen that Rotation 6 gave consistently lower yields than Rotation 8 in every year, showing unmistakably the existence of appreciable differences in soil fertility. It will, therefore, be safer to use Rotation 8 in preference to Rotation 6 for purposes of comparison. For comparison with Rotations 3, 4 and 7 one further advantage would be that they all started in the same year as Rotation 8.

The differences in mean yield from Rotation 8 are shown for each year in Table XI. The last column gives the critical difference calculated from the fluctuations within fields for the year as a whole. A more precise value may be obtained when necessary from the standard errors of the rotations concerned given in Table IV.

TABLE XI.

Differences in yield of cotton from control B (Rotation 8).

Year	1	2	3	4	5	6	7	Critical difference 3 × S. E. of single mean
	Cotton, groundnut	Cotton, *juar*, groundnut	Cotton, cotton, *juar*, groundnut	Cotton, cotton, *juar*	Cotton, *juar*	Cotton every year (control A).	Cotton, cotton, groundnut	
1925-26	+12·6	*J*	+5·8	—4·4	*J*	—19·0	+3·0	11·97
1926-27	G	G	—14·3	—9·4	—7·6	—23·8	+4·4	14·07
1927-28	—1·4	+12·8	*J*	*J*	*J*	—21·8	G	18·63
1928-29	G	*J*	G	+0·8	+3·8	—9·0	+54·0	5·79
1929-30	+56·0	G	+77·8	+36·4	*J*	—1·0	—1·0	23·61
1930-31	G	+29·0	+14·8	*J*	—14·8	—4·8	G	9·42

Rotations 5 and 6 gave lower yields than 8, and need not be considered further. Rotation 4 gave lower or practically the same yield in 3 years and a larger yield in only one season 1929-30; the average difference is not, however, statistically significant. This leaves Rotations 1, 2, 3 and 7.

For Rotation 1, the difference in 1925-26 is +12·6, while the standard error of the difference as calculated directly from the variances for Rotations 1 and 8 is 7·11. This difference cannot, therefore, be considered significant. The difference in 1927-28 is very small (—1·4) and insignificant, but is algebraically in favour of Rotation 8. It will be seen, therefore, that it is the difference in yield in 1929-30 (+56·0) which confers the advantage on Rotation 1. For Rotation 2, on the other hand, the differences in 1927-28 (+12·8 with a S. E. of 4·12) and in 1930-31 (+29·0 with a S. E. of 5·03) are both clearly significant. In the case of Rotation 3, the difference in earlier years, 1925-26, are insignificant (the one in 1926-27 being negative), but the differences in 1929-30 and 1930-31 are definitely significant. For Rotation 7, the differences were negligible in 3 seasons, and significant in only one year, 1928-29.

Comparing the yields in each year for the other rotations we find that all the differences are insignificant except in the season 1929-30 when the yield of Rotation 7 was significantly lower than the yields of Rotations 1 and 3.

Using Rotation 8 as the 'control' and comparing from year to year, we find that Rotation 2 gave consistently better yields in two years, Rotation 3 also gave

better results in two years, but had no advantage in two other seasons, while Rotations 1 and 7 gave appreciably better results in one season each, differences in other seasons being statistically negligible. Differences between Rotations 2, 3 and 1 were negligible throughout, while Rotation 7 gave definitely worse results than these in one season only. Owing to the irregular nature of the yield in Rotation 7, it is, however, not possible to draw any reliable conclusions.

The general position may now be summarised. So far as cotton is concerned, if we ignore initial soil differences we find that Rotations 2 and 3 and possibly 1 constitute a group of rotations which all included groundnuts and which gave on the whole larger yields than Rotation 8 (cotton every year), differences within the group (that is among Rotations 2, 3 and 1) being statistically inappreciable. The differences in the case of Rotations 4, 5 and 6 were insignificant, while the small advantage in favour of Rotation 7 cannot be considered clearly established.

Effect of groundnut and *juar*.

It will be noticed that Rotations 2, 3, 1 and 7 all include groundnut. The question, therefore, arises whether a crop of groundnut confers any beneficial effect on a successive crop of cotton. I have made an attempt to study this question directly by pooling together the results for the different rotations. The relevant data will be found in Table XII. The standard error of the difference in yield has been calculated in each case from the variances of the rotations concerned.

Table XII.

Mean yields of cotton following groundnuts and cotton.

Year	Following a groundnut crop			Following a cotton crop			Mean difference	Standard error of difference
	Rotations	*n*	Mean	Rotations	*n*	Mean		
1925-26	1	5	50·6	6	5	19·0	+31·6	7·20
1927-28	1, 2	10	82·9	6, 8	10	65·8	+17·1	8·79
1928-29	7	5	90·2	6, 8	10	30·7	+59·5	2·18
1929-30	1, 3	10	111·1	4, 6, 8	15	55·6	+55·5	12·15
1930-31	2	5	75·6	3, 6, 8	15	50·0	+25·6	5·59

It will be noticed that the differences in yield were always in favour of a cotton crop succeeding a groundnut crop, and were all statistically significant. The above analysis would appear to indicate that there is a real advantage in using groundnut as a rotation crop with cotton. One reservation is, however, necessary. The data for the yield following a cotton crop are predominantly based on Rotations 6 and 8, and any intrinsic deficiency in soil fertility of the fields under these two rotations would affect the results in favour of groundnut. It is worth while remarking at this stage that we have already found some evidence to show that the field under Rotation 6 was intrinsically poor.

We can in the same way study the effect of *juar* on a succeeding cotton crop. The necessary data are given in Table XIII.

TABLE XIII.

Mean yields of cotton following juar.

Year	Following a *juar* crop			Following a cotton crop			Mean difference	Standard error of difference
	Rotations	*n*	Mean	Rotations	*n*	Mean		
1926-27	5	5	39·00	3, 4, 6, 7, 8	25	36·22	+2·78	7·00
1928-29	4, 5	10	39·00	6, 8	10	30·70	+8·80	2·86
1930-31	5	5	31·80	3, 6, 8	15	49·93	—18·13	3·15
Total .	..	20	37·45	..	50	39·23	—1·78	4·82

The difference in 1926-27 is inappreciable, that in 1928-29 is +8·80 (with standard error 2·86) in favour of *juar* and on the verge of significance, while the difference in 1930-31 is —18·13 (with standard error 3·15) which is against *juar* and statistically significant. Combining the data for all the three seasons we get a small difference —1·78 (with standard error 4·82) against *juar* which is, however, negligible. We must conclude that *juar* did not exert any appreciable influence on the yield of a succeeding cotton crop.

YIELDS CORRECTED FOR SEASONAL EFFECTS.

We shall now try to eliminate the seasonal effect by constructing some kind of seasonal index numbers of yield. One way of doing this would be to take the mean

yield over the whole period for Rotation 6 (cotton every year) as the standard of comparison (=100). The actual yield for Rotation 6 in any particular year can then be expressed as a percentage of the mean yield, and such percentages may be used as seasonal index numbers. The calculated figures are given in column 2 of Table XIV.

TABLE XIV.

Seasonal index numbers for yield of cotton.

Year (1)	Based on Rotation 6 (2)	Based on all rotations (3)
1923-24 . . .	74·2	76·9
1924-25 . . .	79·2	66·8
1925-26 . . .	59·2	77·7
1926-27 . . .	71·1	75·5
1927-28 . . .	172·7	143·4
1928-29 . . .	78·6	95·1
1929-30 . . .	134·7	148·9
1930-31 . . .	130·3	106·0

Instead of using the results for Rotation 6 only, we can use the mean yield for all rotations for the whole period as the standard (=100), and construct the seasonal index numbers from the mean yield of all rotations for different years. These figures are given in column 3 of Table XIV. It will be seen that the two series are roughly parallel, although there are considerable differences in particular years. We have actually used the mean index numbers based on all rotations (col. 3, Table XIV). In order to eliminate the seasonal effect we must divide the yields of all fields in any particular year by the corresponding seasonal index number, or multiply by its reciprocal.

We have already seen that Rotations 1, 2 and 3 are more important than Rotations 4, 5 or 7 so far as the yield of cotton is concerned. The mean yield of cotton corrected for the seasonal effect for these three rotations and for the two 'controls' 6 and 8 are given in Table XV.

TABLE XV.

Mean yields of cotton corrected for seasonal effect (in lbs. per 1/10th acre).

Year	1923-24	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31	Mean
1. Cotton, groundnut.	41·3±3·32	G	65·1±8·13	G	52·9±7·66	G	67·3±8·95	G	56·65±4·20
2. Cotton, *juar*, groundnut.	..	82·8±6·84	*J*	G	63·0±2·02	*J*	G	71·2±4·59	72·33±3·40
3. Cotton, cotton, *juar*, groundnut.	..	..	56·4±4·47	42·7±4·06	*J*	G	81·8±5·57	57·8±3·87	61·56±3·84
6. Cotton every year (control A).	31·2±4·50	37·8±4·80	24·4±4·39	30·0±3·62	38·8±1·88	26·4±2·01	29·0±3·13	38·4±2·16	32·33±1·44
8. Cotton every year (control B).	..	..	49·0±4·36	61·8±5·97	53·0±2·12	38·2±1·16	29·8±2·65	43·8±1·36	45·93±2·29

The corresponding analysis of variance is given in Table XVI.

TABLE XVI.

Analysis of Variance : yields of cotton corrected for seasonal effect.

Rotation	Between years		Within years		Total		Ratio of variance	
	D. F.	Variance	D. F.	Variance	D. F.	Variance	Observed	5 per cent.
1	3	722·08	16	273·03	19	341·30	2·64	3·24
2	2	494·90	12	119·80	14	173·40	4·13	3·89
3	3	1107·65	14	101·25	17	278·85	10·94	3·34
6	7	169·43	32	64·19	39	83·08	2·64	2·39
8	5	635·80	24	58·13	29	157·72	10·90	2·62

Comparing the ratio of variance given in Table V (actual yields) and Table XVI (yields corrected for seasonal effect), we notice the substantial reduction in the fluctuations due to seasonal effects for Rotations 2, 3 and 6, while for Rotation 1 it has become insignificant. With the help of the seasonal index numbers we have, therefore, succeeded in getting rid of the greater part of the seasonal differences in yield.

The superiority of Rotations 2, 3 and 1 over 6 remains definitely significant. Rotations 2 and 3 also give appreciably larger yields than Rotation 8, but the difference between Rotations 1 and 8 is only just on the verge of significance. The position of 1 is thus rendered slightly doubtful. The difference in yield between the two 'controls' 6 and 8 is seen to be definitely significant. This result confirms the existence of intrinsic differences in soil fertility.

FERTILITY INDEX.

We may study the effect of rotation from a different point of view. One rotation may be considered superior or inferior to another according as it succeeds in improving (or maintaining unimpaired) the fertility of the soil, or leads to soil

deterioration as compared to the other. In order to study this effect, we may construct fertility indices by dividing the yield in the same field in different years by the yield in the basic year. Unfortunately owing to rotations having been started in different years in the present series of experiments, the basic year will differ for different rotations. This cannot, however, be helped.

The fertility indices for actual yields of cotton for Rotations 1, 2, 3, 6 and 8 with their standard errors are shown in Table XVII, and the analysis of variance in Table XVIII.

TABLE XVII.

Fertility indices for cotton (actual yields).

Year	1923-24	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31	Average
1. Cotton, groundnut	—	—	166·6±27·13	G	239·4±17·06	G	330·4±57·70	G	245·5±31·4
2. Cotton, *juar*, groundnut.	—	—	*J*	G	169·8±22·50	*J*	G	143·8±23·00	156·8±15·78
3. Cotton, cotton, *juar*, groundnut.	—	—	—	83·0±5·29	*J*	G	279·8±10·73	141·8±17·98	181·3±24·58
6. Cotton every year (control A).	—	113·2±18·53	78·8±6·69	105·2±21·51	250·2±34·93	111·2±10·52	185·8±8·76	198·2±22·69	147·5±11·87
8. Cotton every year (control B).	—	—	—	126·0±14·11	204·8±12·96	98·2±8·49	114·6±14·83	126·8±12·77	144·08±9·20

TABLE XVIII.

Analysis af Variance : fertility of indices for cotton (actual yields).

Rotation	Between years		Within years		Total		Ratio of variance	
	D. F.	Variance	D. F.	Variance	D. F.	Variance	Observed	5 per cent.
1	2	33675·05	12	11650·30	14	14795·93	2·88	3·89
2	1	1690·0	8	2591·0	9	2491·0	<1·0	..
3	2	42650·0	10	893·8	12	7853·3	47·4	4·10
6	6	18894·8	28	1928·6	34	4934·5	9·80	2·45
8	4	8583·0	20	824·2	24	2117·33	10·4	2·87

In the case of Rotation 1 there is an apparent increase in the fertility index from year to year, while the indices vary irregularly in other cases.

It will be noticed from Table XVIII that the seasonal effect is very pronounced, and some of the irregularities must be ascribed to this factor. It will, therefore, be desirable to work with the yields corrected for the seasonal effect. Doing this we obtain the fertility indices (corrected for the seasonal factor) given in Table XIX, and the corresponding analysis of variance given in Table XX.

TABLE XIX.

Fertility indices for cotton (yields corrected for seasonal effect).

Year	1924-25	1925-26	1926-27	1927-28	1928-29	1929-30	1930-31	Average
1. Cotton, groundnut . .	G	164·4±26·72	G	135·0±25·69	G	170·6±30·00	G	156·67±15·30
2. Cotton, *juar*, groundnut .	—	*J*	G	79·6±10·71	*J*	G	90·8±14·63	85·2±8·74
3. Cotton, cotton, *juar* .	—	—	85·0±9·85	—	G	146·6±7·25	107·8±17·16	117·5±9·81
6. Cotton every year (control A).	128·0±20·94	77·4±6·48	105·6±21·31	133·4±18·44	88·6±8·48	94·8±4·70	135·0±15·99	109·03±6·40
8. Cotton every year (control B).	—	—	129·8±15·68	110·4±7·10	80·4±7·39	62·2±5·95	92·8±9·39	95·12±6·22

TABLE XX.

Analysis of Variance for Fertility Indices for Cotton (yields corrected for seasonal effect).

Rotation	Between years		Within years		Total		Ratio of variance	
	D. F.	Variance	D. F.	Variance	D. F.	Variance	Observed	5 per cent.
1	2	1809·0	12	3791·2	14	3508·0	<1·0	..
2	1	313·6	8	821·0	9	764·62	<1·0	..
3	2	3937·0	10	713·0	12	1250·33	5·52	4·10
6	6	2716·9	28	1159·88	34	1434·6	2·35	2·45
8	4	3427·25	20	474·85	24	966·96	7·22	2·87

It will be noticed from Table XX that for Rotations 1, 2 and 6 the seasonal differences are no longer significant. In the case of Rotation 3 also it is so much reduced that the observed ratio of variances (5·52) is not much greater than the just appreciable (5 per cent.) ratio (4·10). The only exception is Rotation 8, for which the seasonal variation is definitely significant.

In Rotation 1 the corrected indices do not confirm the progressive improvement in fertility shown by the raw indices. The results with the raw indices must therefore be ascribed to the seasonal effect. Rotation 2 shows a lowered index of fertility (85·2) while Rotation 3 shows a small improvement (117·5), but neither of these effects are definitely significant.

'Control' Rotation 6 (cotton every year) also shows a small improvement (109·0) on the whole, which, however, is quite negligible in comparison with its standard error. It is clear, however, that Rotation 6 does not show any progressive loss of fertility. 'Control' Rotation 8 (cotton every year), on the other hand, shows unmistakable evidence of progressive soil deterioration. In fact with the exception of the year 1930-31, there is a steady decline in the fertility index (129·8, 110·4, 80·4 and 62·2) in successive years. As I have already remarked, this is also the one single case in which the seasonal effect is very definitely significant. The progressive deterioration of soil fertility in the case of Rotation 8 is thus clearly brought out.

SUMMARY OF ANALYSIS.

We may now summarise the results of the statistical analysis. The soil fertility levels of the different fields under different rotations were probably not equal.

Seasonal effects were also very strong, and masked the rotational effects in most cases. In control B (Rotation 8, cotton every year) there was distinct evidence of progressive exhaustion of the soil under successive crops of cotton. Such exhaustion was, however, not noticed in control A (Rotation 6, cotton every year), and this was probably due to the originally poor character of the field. Ignoring initial soil differences, Rotations 2 (cotton, *juar*, groundnut), 3 (cotton, cotton, *juar*, groundnut), and possibly 1 (cotton, groundnut) on the whole gave larger yields of cotton.

An attempt was made to eliminate the effect of seasons and of initial differences in soil fertility by constructing correcting indices. The corrected yields failed to show significant differences between the rotations, but brought out the progressive deterioration of the field under successive cotton crops in Rotation 8. The method of using correcting indices is, however, purely empirical in character, and the results cannot be considered conclusive.

Comparing the pooled results for different rotations it was found that the yields were always in favour of a cotton crop succeeding a crop of groundnut but not of *juar*. The balance of evidence is thus distinctly in favour of the view that a crop of groundnut (but not of *juar*) exerts a beneficial influence on a succeeding crop of cotton. This fact, however, cannot be established with scientific precision owing to defective planning of the experiments.

Plot design for rotational experiments.

In view of the importance of the question, a few suggestions are made on the design of rotation experiments. In order to reach valid results in rotational experiments, the plots must be arranged in such a way that it would be possible to eliminate (*a*) the soil differences, as well as (*b*) the effect of different seasons. A Latin Square (or Randomised Block) arrangement is essential for (*a*), while a complete cycle of years is necessary for (*b*). Finally (*c*) there must be a sufficient number of replications in order to attain the desired degree of precision.

A concrete illustration may make clear the principles involved in the construction of such designs. Let us consider the following 5 rotations :—

Rotation 1 : Cotton every year (C, C, C, C, C, C,.....)

Rotation 2 : Cotton, groundnut alternately (C, G, C, G, C, G, C).

Rotation 3 : Cotton, *juar* alternately (C, *J*, C, *J*, C, *J*, C, *J*, C).

Rotation 4 : Cotton, groundnut, *juar* (C, G, *J*, C, G, *J*, C, G, *J*).

Rotation 5 : Cotton, *juar*, groundnut (C, *J*, G, C, J, G, C, *J*, G).

The rotation schemes for 6 years are shown in Fig. 1. In the seventh year all the plots will again receive cotton.

Rotations	Seasons						
	1	2	3	4	5	6	7
1	C	C	C	C	C	C	C
2	C	G	C	G	C	G	C
3	C	*J*	C	*J*	C	*J*	C
4	C	G	*J*	C	G	*J*	C
5	C	*J*	G	C	*J*	G	C

C=Cotton

G=Groundnut

J=*Juar*

1st Year

All cotton

2nd Year

J	G	C	G	*J*
C	*J*	G	*J*	G
G	C	G	*J*	*J*
G	*J*	*J*	C	G
J	G	*J*	G	C

3rd Year

C	*J*	C	C	G
C	G	*J*	C	C
J	C	C	G	C
C	C	G	C	*J*
G	C	C	*J*	C

4th Year

J	C	C	G	C
C	C	C	*J*	G
C	C	G	C	*J*
G	*J*	C	C	C
C	G	*J*	C	C

5th Year

C	G	C	C	*J*
C	*J*	G	C	C
G	C	C	*J*	C
C	C	*J*	C	G
J	C	C	G	C

6th Year

J	*J*	C	G	G
C	G	*J*	*J*	G
J	C	G	G	*J*
G	*J*	G	C	*J*
G	G	*J*	*J*	C

Fig. 1. A scheme for a six-year rotation experiment.

The accumulated effect of the rotations will be ready for analysis after the seventh year of the experiment. It will, however, be desirable to conduct the experiment over two (or more) complete cycles; with the present design, 13 years will be the desirable minimum period. The precision of the experiment can be considerably increased if two or more Latin Squares are laid out for the experiment with different random arrangements of the plots (but with the same sequence of rotations).

When possible an attempt may also be made to balance the seasonal effects by using a suitable number of Latin Squares simultaneously. Consider the following four rotations :—

(1) Cotton every year (C, C, C, C).

(2) Cotton, *juar* alternately (C, *J*, C, *J*, C).

(3) Cotton, groundnut alternately (C, G, C, G, C).

(4) Cotton, *juar*, *juar*, groundnut (C, *J*, *J*, G, C).

It will be noticed that one whole cycle will be completed in four years. We may, therefore, use simultaneously four separate Latin Squares (each divided into 4×4 plots), each phase of the cycle being represented by one Latin Square. One set of randomized lay-out constructed by Mr. Subhendu Sekhar Bose of the Statistical Laboratory, Presidency College, Calcutta, is given in Fig. 2. It is possible, of course, to construct designs of the same type for other rotations, and we are prepared to supply suitable designs on receipt of detailed specifications.

Rotation	Seasons				
	1	2	3	4	5
1	C	C	C	C	C
2	C	*J*	C	*J*	C
3	C	G	C	G	C
4	C	*J*	*J*	G	C

C=Cotton
G=Groundnut
J=*Juar*

1st Year

(1) (2)

All cotton	G G *J* C *J* C G G C *J* G G G G C *J*
J G *J* C *J* C *J* G C *J* G *J* G *J* C *J*	*J* C C C C C *J* C C C C *J* C *J* C C

(3) (4)

2nd Year

(1) (2)

J G *J* C *J* C *J* G C *J* G *J* G *J* C *J*	All cotton
J C C C C C *J* C C C C *J* C *J* C C	G G *J* C *J* C G G C *J* G G G G C *J*

(3) (4)

3rd Year

(1) (2)

J C C C C C *J* C C C C *J* C *J* C C	*J* G *J* C *J* C *J* G C *J* G *J* G *J* C *J*
G G *J* C *J* C G G C *J* G G G G C *J*	All cotton

(3) (4)

4th Year

(1) (2)

G G *J* C *J* C G G C *J* G G G G C *J*	*J* C C C C C *J* C C C C *J* C *J* C C
All cotton	*J* G *J* C *J* C *J* G C *J* G *J* G *J* C *J*

(3) (4)

Fig. 2.[5] A scheme for a four-year rotation experiment.

ANTHESIS AND POLLINATION IN RAGI, *ELEUSINE CORACANA* (GAERTN.), THE FINGER MILLET.

BY

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(Received for publication on 28th March 1933.)

(With Plate XXIV.)

Pollination studies are a necessary preliminary in the improvement of any crop, and especially so for the millets. Such observations are as necessary to the study of pure lines, and their maintenance as such, as in the devising of successful hybridization schemes.

Previous work.

The mode of pollination in this finger millet has not received much detailed attention. Knuth in his "Hand Book of Flower Pollination" [Ainsworth Davis, 1909] makes a passing reference to the self-pollination that takes place in *ragi*. Badami [Rangaswami Ayyangar, 1925] devised a method of effecting successful emasculation in the delicate flowers of this millet by enclosing the flowers in a moist chamber which enabled the anthers to emerge intact. The only detailed record of anthesis is that by Gokhale, Bhosekar and Patwardhan [1932] at Poona.

Material.

Floral observations were in progress on the following material from 1927 onwards :—

Family No.	Earhead character	No. of plants under observation	No. of seasons
E. C. 297	Purple-Topcurved	10	5
E. C. 374	Purple-Incurved	5	3
E. C. 364	Purple-Open	5	3
E. C. 1212	Purple-Fist-like	2	1
E. C. 1000	Green-Incurved	2	1

The emergence of the flag and the panicle.

The distichous and telescopic arrangement in the leaf-sheaths of this millet gives greater prominence to the leaf blades. This prominence is such that the flag, the ultimate leaf of the stem and the fore-runner of the earhead, is not in marked distinction from the rest of the leaves, as is the case in many other cereals. As the sheath of this flag is to be the container of the earhead, its appearance was watched. The difficulty of spotting the ultimate leaf is very real. The flag (the blade of the ultimate leaf) takes about a week to emerge. When the blade is about half emerged, there is intensive growth activity in the plant resulting in the emergence of the earhead, close after the complete emergence of the flag. The earhead proper emerges rapidly in two to three days and for the complete emergence of the inflorescence with its stalk and the consequent cessation in growth, it takes about ten days.

The spike.

Unlike other cereals, this 'finger millet' consists of a whorl of fingers, the spikes of the inflorescence. These fingers vary from four to six in primary heads and as the structure of each of these is similar to that of the others, floral observations on one of these spikes is fairly typical of that of the sister spikes comprising the earhead. The only difference is that introduced by the thumb, the spike inserted a little lower down the main spike-whorl. This thumb is usually the most vigorous of the spikes, the vigour manifesting itself in a tendency for the spike to branch, each branch carrying spikelets on it. This prolificness in spikelets notwithstanding, the thumb makes up by more rapid flowering and keeps pace with the normal flowering range of the other spikes.

The spikelet.

Per spikelet, the number of flowers varies from 4 to 6, 4 and 5 being the commonest; two, three and seven-flowered spikelets are comparatively rare. Occasionally a sigle-flowered spikelet occurs. There is no selective distribution of the major groups in these spikelets, but there is a tendency for the fewer-flowered spikelets to occur in the lower half of the finger. It is possibly this factor that influences the sparseness of grains in this region.

The shape of panicle, its colouration and the season in which the earhead is sown had no appreciable effect on this general incidence.

In each spikelet the end glumes are smaller than the basal ones resulting in a slight difference in the size of the grains produced. The following measurements of the glumes explain this point:—

Glume No.	Length and width in mm.
I	4·5 by 2·0
II	5·5 by 2·0
III	5·0 by 2·5
IV	5·0 by 2·0
V	4·75 by 2·0
VI	4·25 by 1·5
VII	4·0 by 1·5

It is worth recording the fact that whereas all the flowering glumes have an extra nerve at each of their edges, such nerves are absent in the two basal sterile glumes.

ANTHESIS.

An earhead takes seven to eight days to complete its flowering. Occasionally a day or two more may be taken by flowers with a larger number of spikelets.

Observations on the period of anthesis in a day have been made on many types of earheads in various seasons and are given below:—

SUMMARY OF ANTHESIS.

Shape of earhead	No. of flowers opened during all the days of the anthesis between the hours						Total
	1—2 a.m.	2—3 a.m.	3—4 a.m.	4—5 a.m.	5—6 a.m.	6—7 a.m.	
Top-curved	..	756	888	591	107	16	2,358
,,	..	536	1,331	165	8	..	2,040
,,	..	210	1,233	241	10	..	1,694
,,	..	889	1,286	819	..	..	2,994
,,	..	720	1,020	425	..	..	2,165
,,	..	892	1,352	230	9	..	2,483
	..	4,003	7,110	2,471	134	16	13,734

SUMMARY OF ANTHESIS—*contd.*

Shape of earhead	No. of flowers opened during all the days of the anthesis between the hours						Total
	1—2 a.m.	2—3 a.m.	3—4 a.m.	4—5 a.m.	5—6 a.m.	6—7 a.m.	
In-curved	..	188	385	401	47	..	1,021
,,	..	84	829	308	60	..	1,281
,,	..	..	870	328	..	..	1,198
,,	..	6	1,100	553	9	..	1,668
,,	..	612	587	495	166	..	1,860
,,	..	473	1,082	467	..	..	2,022
,,	..	847	923	505	..	..	2,275
	..	2,210	5,776	3,057	282	..	11,325
Fist-like	..	162	679	697	..	..	1,538
,,	..	311	921	748	..	..	1,980
	..	473	1,600	1,445	..	..	3,518
Open	472	370	9	..	..	..	851
,,	970	340	6	..	..	..	1,316
,,	827	358	..	..	..	..	1,185
,,	982	338	..	..	..	..	1,320
,,	2,088	722	..	..	..	..	2,810
	5,339	2,128	15	..	..	..	7,482

The following figures summarise the above readings :—

Opening	Earheads	
	Open (5)	Curved (15)
	5,339	..
1—2 a.m.	2,128	6,686
2—3 „	15	14,486
3—4 „	..	6,973
4—5 „	..	416
5—6 „	..	16
6—7 „		
	7,482	28,577

The flowering is continuous and rapid and for the sake of convenience has been arranged in hourly units.

In the curved earheads (Top-curved, In-curved and Fist-like), anthesis was confined to the period 2 to 5 a.m. with odd flowers beyond this period. In panicles with open fingers the period was clearly an hour earlier, being between 1 and 3 a.m. The period was compressed and the anthesis very quick, crowded and in much more rapid succession than in the case of curved earheads. Among the curveds there was a tendency for flowering to be a bit more concentrated with the advance in flowering, it being heaviest towards the end in the Fist-like heads, less so in the In-curved and the biggest initial flush in the Top-curved, a tendency explicable in terms of the general freedom enjoyed by the spikelets consequent on the various types of crowding and curving.

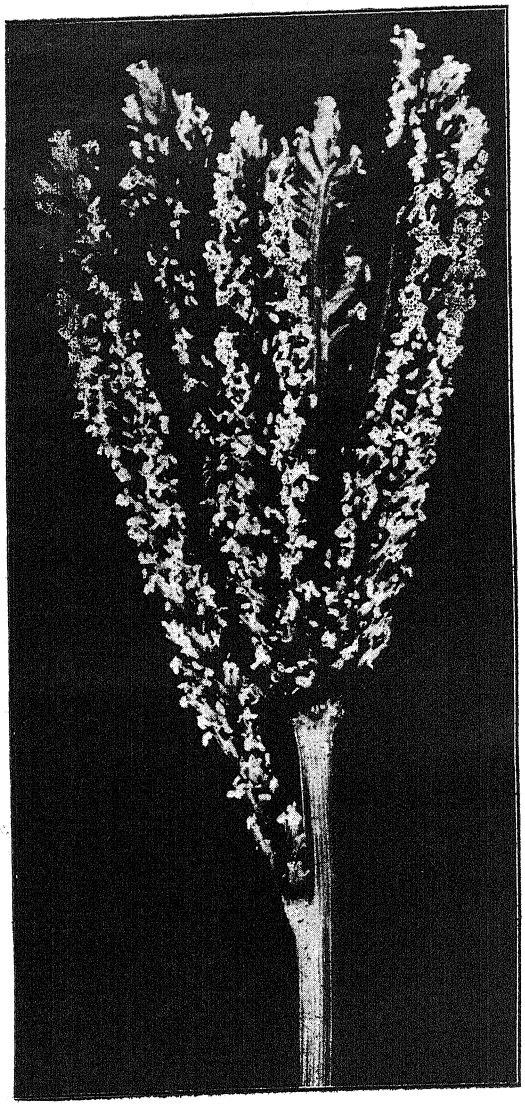
Observations made on *Eleusine aegyptiaca*, the wild grass, common round about Coimbatore, with its short flat radiate fingers show that its flowers open in the course of an hour round about 1 a.m. The tiny heads complete flowering in about a week, the biggest flush being in the first half of this period. The close affinity in floral habit of this wild Eleusine to the open-fingered panicles in cultivated *ragi* is most interesting.

Order of anthesis.

Intensive observations have been made on the order of the opening of flowers in a spike. The general tendency of the flowers is to open and progress from the

PLATE XXIV.

ELEUSINE CORACANA GAERTN.



Earhead in flower.

top to the bottom, in a finger. In a spikelet the order is reversed and proceeds from the bottom to the top, from the bigger to the smaller flower. It can be broadly said that in a spikelet one flower opens per day, though the close distichous arrangement of the flowers in the spikelet often results in two flowers, one on each side, opening the same day. This occurs in spikelets with the larger number of flowers, so much so that it could be generalised that an average spikelet of 5 flowers completes its flowering in five days. The finger, consisting on an average of about 80 spikelets, takes about eight days to complete its flowering. It is therefore obvious that floral openings occur and progress simultaneously in a number of spikelets at a time, the waves progressing downwards. This progression, in a whorl of spikelets, is markedly visible in the mass of lilac-coloured anthers that each day stream down the periphery of an earhead in flower (Plate XXIV).

Detailed observations have been made on individual flowers and their opening and closing. These observations have been done on over 60 flowers in many seasons and varieties. The following programme represents a typical reading in this varied set of occurrences.

	a. m.	
	Hour	Minutes
Glumes begin to open	3	0
Anthers and stigma just visible	3	3
Anthers begin to elongate	3	7
Stigma exserts through the glumal slits	3	13
Glumes open completely	3	14
Anthers come in a level with the top of the glume and dehisce	3	15
Anthers fall out	3	20
Glumes close between 7 and 8-30 a.m. according to the weather.		

It will be noticed how quick the anthesis is and how favourable the conditions are for self-pollination. Between adjacent earheads, there are possibilities of chance cross-pollination, though the experience at the Millets Station does not make this any more than one per cent. in nature. Earheads brought together in artificial contact have crossed fairly wide, the crossing being in some cases as much as 20 per cent.

Cliestogamous flowers of the sort mentioned by Gokhale have not been met with. Stray flowers have been noticed with a restraint on the free exsertion of the filaments, which, when disengaged with a pin, proved normal.

Receptivity of stigma.

The stigma is effectively receptive only for a very short period after its emergence from the glumes. A number of artificially emasculated flowers were pollinated from 1 to 8 hours after the usual time of dehiscence and the results show a weakening in setting of grain with each hour beyond the normal, until after 5 hours, *viz.*, 8 a.m. all pollinations proved ineffective.

Crossing technique.

The emasculation of this flower, though very difficult, is quite a practical proposition. With experience flowers likely to open on the day wanted can easily be spotted on the previous evening, and with a pair of fine forceps the glumes are opened out and the anthers removed. Prior to this operation the rest of the spikelets in the spike and other flowers in the spikelet, are removed with scissors. The flower operated on is inserted into a tube the mouth of which is partially plugged with a bit of cotton wool. Early in the morning the selected pollen is dusted on to the stigmas protruding from the gaping glumes. Delicate as this process is, successes ranging from 60 to 90 per cent. have attended the many manipulations made in the early days of hybridization work at the Millets Station. With increased experience and a knowledge of the inheritance of the more obvious characters, of which purple pigmentation is one, desirable parents can be chosen and crossed with each other by what is commonly known at the Millets Breeding Station as the "Contact" method [Rangaswami Ayyangar, 1932]. By this method it has been possible to clip off all except a single finger or part thereof in certain mother varieties and to pollinate them by contact with the desired male earhead—a sort of natural pollination through the agency of man, with the usual precautions to keep off pollen not desired. This has been very successfully practised with all millets including Sorghum. The profuse anthers and stigmas in juxta-position to each other afford most convenient opportunities for a controlled crossing by natural methods, so much so, that when the earhead with the recessive character is sown the requisite first generation plants are spotted without difficulty, the rest being pulled out and the F_1s selfed. By this simple method an experienced breeder can readily increase his wealth of hybrid populations towards a quick sifting of desirable characters.

SUMMARY.

Ragi flowers open from 1 to 4 a. m. Earheads with open fingers open earlier than those with curved fingers. The period of anthesis in the flower is very short and conduces to self-pollination but occasional cross-pollination can occur in nature.

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NOTES

IMPORTATION OF ELM TREES AND CONIFERS INTO ENGLAND.

The Ministry of Agriculture and Fisheries, England, has by arrangement with the Forestry Commissioners issued the order reproduced below called the "Importation of Elm Trees and Conifers (Prohibition) Order of 1933" to prohibit the landing in England and Wales of the eight genera of the Order Pinaceae specified in the Schedule. The order which will come into force on the 1st December, 1933, requires that the health certificates prescribed under the Importation of Plants Order of 1933 to accompany consignments of living plants imported into the country must include a statement to the effect that the consignment does not contain any plants of the genera now prohibited. An order in similar terms applicable to Scotland is being made by the Department of Agriculture for Scotland.

STATUTORY RULES AND ORDERS, 1933, No. 1011.

Destructive Insect and Pest, England.

THE IMPORTATION OF ELM TREES AND CONIFERS (PROHIBITION) ORDER OF 1933.
DATED OCTOBER 24, 1933.

(*D. I. P. 570.*)

The Minister of Agriculture and Fisheries by virtue and in exercise of the powers vested in him under the Destructive Insects and Pests Acts, 1877 to 1927, (*a*) and of every other power enabling him in this behalf, and by arrangement with the Forestry Commissioners under Section 3 (2) of the Forestry Act, 1919, (*b*) order as follows :—

Commencement.

1. This order shall come into operation on the first day of December, nineteen hundred and thirty-three.

Prohibition of Importation of Elm Trees and certain Conifers.

2.—(1) For the prevention of the introduction of diseases and pests injurious to elm trees and forest trees, the landing in England or Wales from any country

(*a*) 40-1 V. c. 68, 7 E. 7. c. 4 and 17-8 G. 5. c. 32.
(*b*) 9-10 G. 5. c. 58.

other than Scotland, Northern Ireland, the Irish Free State, the Isle of Man or the Channel Islands of any living plant of any of the genera mentioned in the Schedule to this Order is hereby prohibited.

(2) In this Article "plant" includes tree and shrub and the roots, layers, cuttings and other parts of a plant.

Restriction on Importation of Plants.

3. The certificates prescribed in Article 4 of the Importation of Plants Order of 1933 (*c*) shall, except in the case of a consignment consisting wholly of potatoes, include a statement to the effect that the consignment does not contain any plant of any of the genera mentioned in the Schedule hereto.

Procedure where Plants are landed in contravention of this Order.

4. If plants mentioned in the Schedule hereto are landed in England or Wales in contravention of this Order, they shall forthwith be destroyed or re-exported at the expense of the importer unless they are otherwise disposed of in accordance with the terms of a licence issued by the Minister or by an Inspector, and any person failing to comply with the terms of a licence granted under this Article shall be liable to a penalty not exceeding ten pounds, or, in respect of a second or subsequent offence, to the penalty not exceeding fifty pounds.

Definitions.

5. In this Order:

"Minister" means the Minister of Agriculture and Fisheries;

"Inspector" means an Inspector or other authorised officer of the Ministry of Agriculture and Fisheries;

"Importer" includes any person who, whether as owner, consignor or consignee, agent or broker, is in possession of, or in anywise entitled to the custody or control of the article.

Revocation of Order.

6. The Importation of Elm Trees (Prohibition) Order of 1926 (*d*) is hereby revoked, provided that such revocation shall not—

(*i*) affect the previous operation of such Order, or anything duly done or suffered under such Order; or

(*ii*) affect any penalty incurred in respect of any offence committed against such Order, or any legal proceedings in respect of any penalty.

(*c*) S. R. & O. 1933, No. 558.

(*d*) S. R. & O. 1926 (No. 1636), p. 373.

Short Title.

7. This Order may be cited as the Importation of Elm Trees and Conifers (Prohibition) Order of 1933.

In witness whereof the Official Seal of the Minister of Agriculture and Fisheries is hereunto affixed this twenty-fourth day of October, nineteen hundred and thirty-three (L. S.).

H. E. DALE,

Principal Assistant Secretary.

SCHEDULE.

All species of the genus Ulmus.

The following genera of the Order Pinaceae, *viz.*, Abies Larix, Picea, Pinus, Pseudotsuga, Sequoia, Thuja and Tsuga.

IMPORTATION OF FRUITS INTO INDO-CHINA.

The following extracts from a translation of the Decrees issued by the Governor General of Indo-China dated the 8th March, and 6th July 1932 regarding the importation of fruits into Indo-China are published for general information :—

Article No. 1.—The importation into Indo-China is authorised of the fruits enumerated in Article No. 2 below if they come from a country which is not declared as infected by the fruit fly (Ceretatis capitata) subject to the condition that such fruits should be accompanied by a certificate from the Phyto-pathological (Plant-Health) Service of the country of origin, and bearing the visa of the administrative authority for the French Colonies or of the French Consular Agent abroad to the effect that the fruits are immune from the attack of the Mediterranean fruit fly (Ceretatis capitata).

The Indo-China Sanitary Police Service may, however, whenever it is considered necessary, inspect the imported fruits and forbid their entry if the inspection shows that they are attacked by the fruit fly.

Article No. 2.—The fruits referred to above are as follows :—

Achras sapota (sapodilla), Anona muricata (Soursop), Artocarpus incisa (Bread fruit), Averrhoa caramlolda, Carica papaya (papaw or papaya fruit), Citrus bigaradia (Seville orange), Citrus indica (Citron), Citrus limonum (lemon), Kumquat (fortunella japonica).

Citrus noblis (mandarin orange and its hybrids), Citrus aurantium (the bitter orange and its varieties), Citrus japonica (the Chinese orange), Citrus sinensis (the sweet orange and its varieties), Citrus decumana (shaddock, grape fruit), Eriobotrya japonica (Japanese medlar), Garcinia mangostana (Mangosteen), Litchi sinensis (Litchee), Mangifera indica (mango), Perses gratissima (Avocadotor pear), Psidium Guajava (Guava), Prunus armeniaca (apricot), Prunus persica (peach), Prunus domestica (plum), Pyrus communis (pear), Pyrus malus (apple).

Article No. 3.—The importation into Indo-China of the fruits enumerated in Article 2 is strictly forbidden when they come from the following countries which are recognised as being infested by the Mediterranean fruit fly (*Ceretatis capitata*) :—

Hawaii, Spain, Italy, Sicily, Greece, Malta, Turkey, Syria, Palestine, Egypt, Tripoli, Tunis, Algiers, Cape Colony, Natal, British East Africa, Madagascar, Brazil, Argentine, Bermuda, Azores, Madeira, Canary Islands, Cape Verd, Dahomey, Nigeria, Congo, Delagoa Bay, Rhodesia, Uganda, Mauritius, Australia (West), New South Wales, Victoria, Queensland, New Zealand and Tasmania.

Article No. 4.—The Secretary General of the Governor-General of Indo-China, the Director of Customs and Taxes and the Director of the Institute of Agricultural Research are entrusted with the execution of the present decrees in so far as each is concerned.

ORIGINAL ARTICLES

PHYSIOLOGICAL INVESTIGATIONS ON WATER-HYACINTH (*EICHHORNIA CRASSIPES*) IN ORISSA WITH NOTES ON SOME OTHER AQUATIC WEEDS.

BY

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(Received for publication on 20th March 1934)

(With Plates XXV—XXX and eight text-figures)

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I. Introduction.

The work, of which an account is given below, was undertaken in 1929 at the instance of Mr. N. F. Peck, I.C.S., the then Collector of Cuttack. Mr. Peck gives

a full account of his campaign against water-hyacinth in his book [Peck, 1930] which was published in 1930. He mentions there (pages 44-67) how the reappearance of water-hyacinth in carefully cleared tanks was puzzling him and his officers and how he and I solved the puzzle.

It was thought necessary to study the water-hyacinth plant with a view to clearing up obscurities in the life-history of the plant, hoping that some indication may be obtained as to the mode of eradication. The Imperial Council of Agricultural Research got interested in the matter and made a grant for continuing the work for two years and a month, without which it would have been impossible to do much.

It is not necessary here to give the details of the morphology of the water-hyacinth plant. Various workers have already pointed out the morphological peculiarities of this water weed. The list of references collected at the end may be consulted for details. It seems desirable to compile a handbook of all the available information on water-hyacinth but that is not the task at present.

It is sufficient to state here that the water-hyacinth, although generally a floating weed, is capable of growth on moist land. It has, therefore, adaptive characters both for water and land. In water it reproduces chiefly by vegetative methods, although it sets seeds quite frequently in these parts at least.

The seedlings germinated in the beginning of the rains grow rapidly under suitable conditions and attain maturity in three months, producing flowers by September. Although the plant flowers almost all the year round, it does not set seed except at the beginning of the cold weather. Capsules are only produced towards the end of September or the beginning of October. It seems to be a question of temperature. In these parts the temperature about this period is 75·7°F. to 85°F. The fact that the spike bends often into water after the flowers wilt, is well known. It is needless to give details of how the capsule matures and seeds are discharged. It is sufficient to state that the capsule bursts and gets detached from the spike on maturity and falls down. The seeds sink to the bottom of the tank and lie buried in the mud.

When the tanks get shallow in summer, the wading birds may disperse the seeds in their feet, as suggested by Brühl and Sengupta [1927], from tank to tank. How the seeds that are exposed to the sun get dried up and thus become fit to germinate when they receive moisture, is described elsewhere.

Evidence as to the period of viability of seeds may be cited here. Some seeds have been stored in the laboratory with a view to finding out the maximum period of viability. From observations in the field, it is certain that seeds remain viable



Fig. 1.

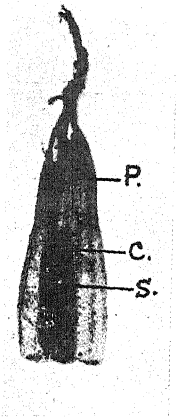


Fig. 2.

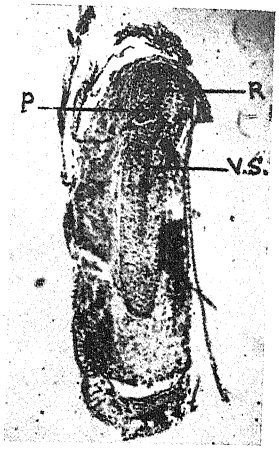


Fig. 3.

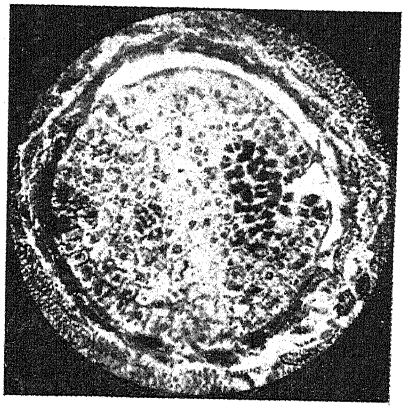


Fig. 4.

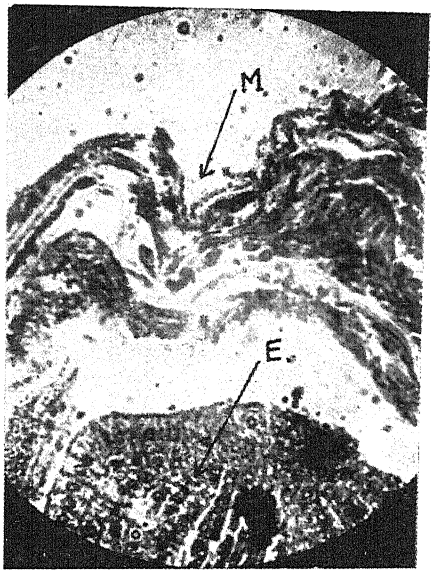


Fig. 6.

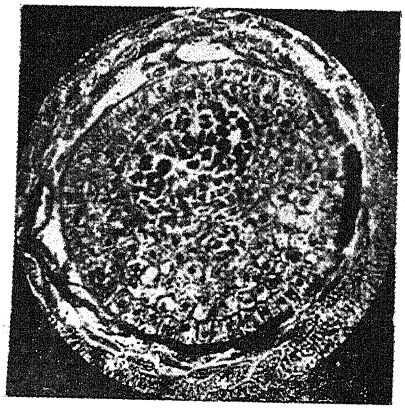


Fig. 5.

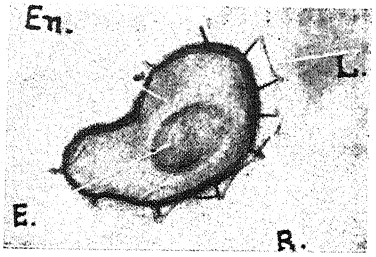


Fig. 7.

(*For explanation see page 429.*)

for five years at least. A tank in the district of Cuttack was cleared during the autumn of 1927, and has been kept free of water-hyacinth since then. Last July (1932) some seedlings were collected from the tank, thus proving that there were some seeds still in the tank which retained their viability for five years. There is, however, the remote possibility that the seeds might have been brought into this tank from outside. This is not likely as the tanks round about had all been cleared.

One imagines that the hard seed-coat and the almost anærobic conditions in the mud at the bottom of the tanks helps the seeds in retaining their viability. As Cole [1932] observed, the concentration of oxygen in mud 10 ft. below the surface of water is only about 0·1 c. c. per litre. This means there is practically no oxygen near the seeds. This presumably helps the seeds to keep the testa intact, and prevents germination. When the seeds get access to oxygen again, they germinate. The germination has already been described and the facts have been corroborated in Burma. [Robertson & Ba Thein, 1932.]

In this brief report only experiments from which definite and interesting results could be drawn have been given.

I am indebted to the Imperial Council of Agricultural Research for their grant. I take this opportunity to express my thanks to Mr. T. C. N. Singh for his very valuable help in preparing the report, and to Mr. B. K. Kar who helped me throughout in the investigation.

II. Dormancy of the seeds of water-hyacinth.

A. Capsule.

The seeds of water-hyacinth are contained in a trilocular capsule and are heavier than water when ripe. When the capsule bursts, the seeds from bent spikes are discharged into the water and sink to the bottom (Plate XXV, figs. 8, 9). The capsule is somewhat like an elongated cone with its base attached to the spike and is contained within the funnel-shaped, accrescent and persistent base of the perianth. It is due to the presence of this perianth that the capsule seems to be a little bigger than what it really is (Plate XXV, figs. 1, 2). The length of the capsule is about 1·2 cm. and the longest diameter is 0·4 cm. The ovary is trilocular containing a total of 50-60 seeds wherever the loculi happen to be completely filled with them; but in many cases the mature seeds are found only in the upper region of the capsule, the basal portion either containing imperfect ones or none at all. This might be due to incomplete fertilization.

B. Seed.

The seeds are minute with an oval base and tapering apex with which it is attached to the wall of the ovary. The dimensions of the seeds are: length 16-21

mm.; diameter (at the thickest zone) 12-16 mm. On the seed-coat there is a number of longitudinal ridges, *viz.* 12 to 15 in number. These are not found in immature seeds, but develop as the latter mature. Although fairly clearly seen on green seeds from undehisced capsule, these ridges, nevertheless, become very prominent in dry seeds. In a transverse section of the seed, the ridges appear to be a skeleton to support the flesh which dries up or decays in time. On the epidermis of the seed, the fleshy layer is still visible as a thin line joining the ridges (Plate XXV, fig. 7). A transverse section of the seed-coat shows an outer and an inner integument. As the ovule is anatropous, the micropyle is not terminal but is situated little towards one side, just below the point of attachment of the seeds to the wall (Plate XXV, fig. 6; Plate XXVI, fig. 4.). The embryo is surrounded by a rather thick endosperm which is covered by a hard-ribbed seed-coat. The embryo agrees in essential details with the seeds of the Pontederiaceae.

C. Dormancy.

Muller as far back as 1883 noted that seeds of *Eichhornia crassipes* require previous drying as a necessary condition for their germination. He failed to germinate freshly harvested seeds. These, however, germinated later when sown again after a period of desiccation. Ludwig demonstrated this in case of the seeds of *Mayaca fluviatilis* [Arber, 1920]. Since then several investigators have studied the subject of delayed germination in water plants, and almost all of them have come to the conclusion that germination is delayed if the seeds are continuously immersed in water and it is accelerated if they are subjected to a period of drying.

Crocker [1907] has shown experimentally that hard seed-coats of the water plant shinder germination by limiting the water supply and that drying followed by soaking seems to induce rupture of the coats and thus to allow growth to begin. Later on Crocker and Davis [1914] confirm the earlier view by a detailed study of *Alisma plantago* where the dormancy of the achenes is due to the mechanical restraint exercised by seed-coats.

This explains the crop of seedlings in large numbers just at the beginning of the monsoons. The intense heat of the summer months totally dries up the soil with the embedded seeds which with the beginning of the rains, get suitable temperature and moisture for their germination. We undertook to investigate the dormancy of the water-hyacinth seeds in the hope of throwing further light on the question. The investigation was divided under three subheads, namely,

(*i*) Period of rest

(*ii*) Resistance of the seed-coat

and (*iii*) Oxygen supply.

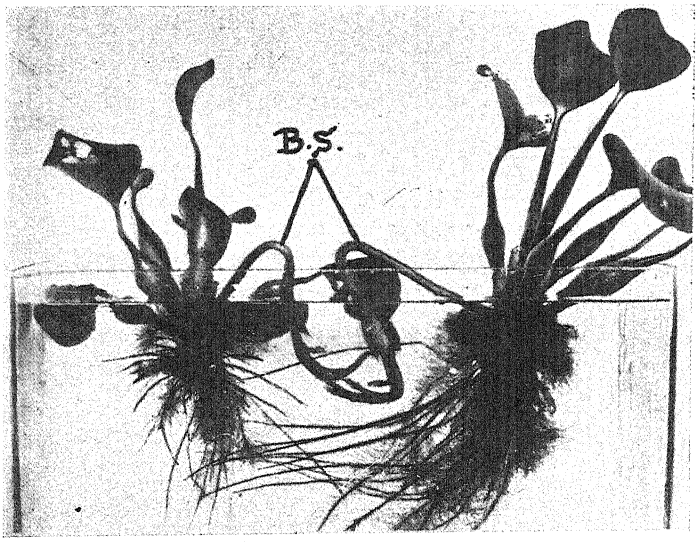


Fig. 1.



Fig. 2.

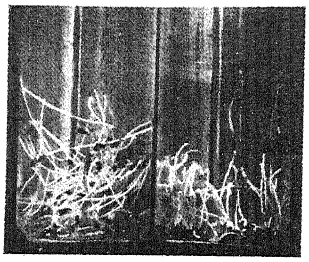


Fig. 3.

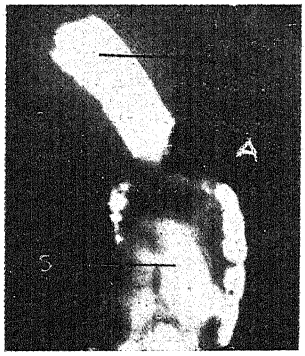
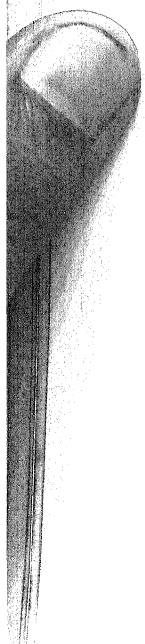


Fig. 4.

(*For explanation see page* 429.)



(*i*) *Period of rest.*—Freshly harvested seeds of water-hyacinth do not germinate. It was thought perhaps the embryo is not fully developed when the seeds are thrown out into the water from the dehisced capsule and the period of rest is necessary for the full development of the embryo. In order to know the condition of the embryo, seeds at all stages of development were collected, namely,

(*a*) green seeds from green capsule

(*b*) mature seeds from green capsule

and (*c*) seeds from dehiscent capsule.

The hard seed-coat was ruptured by means of a needle at the antipodal end to avoid injuring and thus allowing the fixing solution (chromacetic acid) to have access to the embryo. They were then microtomed. As a result of a close examination of our micro-preparations, it has been found that even seeds taken from green mature capsules show a fully developed and complete embryo with plumule, radicle and vascular strand (Plate XXV, figs. 3, 4, 5). The embryo is thus fully formed in mature seeds and this could not, therefore, be the cause of dormancy.

The fully developed seeds collected in November 1930 and dried for two and a half months were kept submerged in water. Side by side some freshly collected seeds during the same month, were submerged in water without any drying. Even after seven months none of these seeds showed any sign of germination. After this period, they were allowed to dry up perfectly for four months by occasionally keeping them in the sun. When watered again almost all of them germinated after three days (Plate XXVI, fig. 3). That drying helps germination was observed by Muller, Ludwig and others. Crocker is of the opinion that drying followed by soaking, causes a considerable per cent. of coats of *Eichhornia* seeds to crack along the fluting. We shall presently see that the testa offers not only mechanical resistance to germination but also prevents the entry of oxygen and thereby retards germination. In fact we shall adduce evidence that insufficiency of oxygen is one of the causes of dormancy of wet seeds. Alternate drying and wetting probably loosens the testa, thereby removing the mechanical resistance as well as facilitating the entry of oxygen.

(*ii*) *Resistance of the seed-coat.*—The seed-coat offers mechanical resistance but is not impervious to water; for when soaked seeds are ruptured, the radicle protrudes out within an hour, which shows that water had got in and the embryo had swollen but the resistance of the testa and the insufficiency of oxygen were preventing further germination. Fig. 1(*a-h*) illustrate the various stages in the development of a seedling from a ruptured seed. In nature, apart from the effect of alternate drying and wetting, the hard testa is probably acted upon by

bacteria or softened by some chemical action. The results of some experiments done with a view to soften the testa to facilitate germination are shown below :

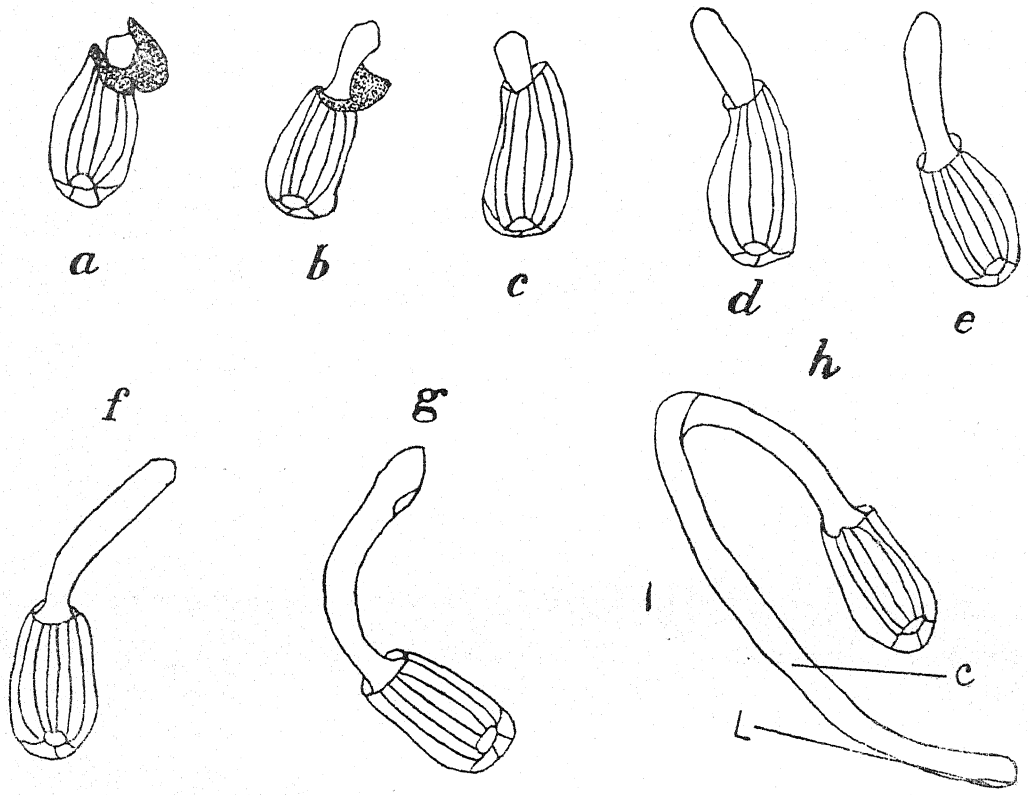


Fig. 1. *a:* A mechanically ruptured seed at the micropylar end showing radicle protruding out; *b:* Ruptured seed showing radicle still more protruding out; *c-g:* Showing further stages of germination of water-hyacinth seed which was made to germinate by mechanical removal of the testa at the micropylar end; *h:* The cotyledon growing out to its limit, and the first leaf coming out. C=Cotyledon; L=First leaf.

Treatment on the seed-coats	No. of seeds treated	No. of seeds germinated	No. of days taken in germination	Percentage germination
Bacterial culture (Hay-bacilli)	8	1	10	12·5
Yeast culture	15	3	15	20
Algal film	6	2	10	33
Dilute H_2SO_4	6	*Nil*	—	*Nil*
Potassium phosphate	16	2	2-10	12·5
Freezing mixture	6	2	8	33
Cracking the testa (Mechanical means)	10	4	8	40
Removing the testa (Micropylar end)	10	10	1-2	100
Removing the testa (Antipodal end)	10	*Nil*	—	*Nil*
Warm water (42°C.)	8	1	7	12·5

(*iii*) *Oxygen supply.*—We have seen that the testa is not impervious to water. It is, therefore, likely that some oxygen gets in with water. The amount of oxygen thus entering must vary according to the depth of water at which the seeds lie, because the concentration of dissolved oxygen varies with depth. Cole [1932] has found that the oxygen concentration decreases rapidly with the increase of the depth, from 4·97 c.c. per litre at the surface to 0·14 c.c. at a depth of 10 ft.

In any case the amount of oxygen entering with the water let in by the testa cannot be sufficient for the resumption of growth by the dormant embryo. Attention was drawn to this possibility of oxygen acting as a limiting factor to germination by the fact that in the field germinating seeds are always found on the surface of the mud and germination takes place after a shower of rain. The function of the rain seems to be, besides supplying the moisture, to expose the seeds which are carried over with mud, and thus give access to oxygen into the seeds. In tanks which had steep sides and filled quickly with water, much fewer seedlings grew up as compared to those which had sloping sides.

In such situations, seeds receive moisture over a large area. Besides moisture, seeds receive oxygen, if they lie on the surface. It is probably for this reason that seeds exposed by rain, germinate rapidly and therefore, seedlings are plentiful after a shower of rain. That want of oxygen has something to do with delayed germination was confirmed by our laboratory experiments. Seeds dipped simply in tap-water, take a long time to germinate, but if ordinary air or oxygen is bubbled through the water, the seeds germinate much quicker as shown in the table below :—

Treatment of the seeds	No. of seeds treated	Percentage germination			
		3rd day	4th day	5th day	7th day
(1) In tap water	9	*Nil*	*Nil*	*Nil*	*Nil*
(2) In air-bubbled water . . .	9	*Nil*	*Nil*	22	44
(3) In oxygen-bubbled water . .	9	*Nil*	22	55	55

When the seeds from the tap-water were removed after 7th day and dipped in oxygenated water, they germinated and behaved similarly as in (3). There may be two possible explanations of this effect of oxygen on germination. Firstly greater concentration of oxygen in the water surrounding seeds ensures sufficient supply of

the gas for the resumption of growth by the resting embryo. Secondly, oxygen may affect the testa in some manner resulting in the softening of the latter and thereby removing the mechanical resistance.

Summary.

The seeds of water-hyacinth remain dormant for at least one season, *i.e.*, November to June and retain their viability for several years. It is found that :—

(*a*) The embryo is fully developed when the capsule bursts, and thus the under development of the embryo cannot be the cause of dormancy, as fully developed embryos are found in green seeds.

(*b*) The seed-coat is the main cause of dormancy :—

(*i*) by offering physical resistance, as the seeds could be made to germinate by rupturing the coat with a scalpel,

(*ii*) by preventing the entry of oxygen, as seeds could be made to germinate by bubbling oxygen through the water in which seeds were soaked.

(*c*) This confirms the suggestion put forward by the author that the mud-covered seeds in the fields do not germinate for lack of oxygen.

III. Germination of seeds of water-hyacinth.

In a preliminary note one has described the various stages of germination in detail. These observations were based on the various stages of seedlings collected in the field under natural conditions. Since then we have been able to germinate a large number of seeds in the laboratory which confirms the previous observations.

It has been noticed that the embryo is fully formed at the time when the seeds are shed from the capsule into the water, and the micropyle is at one end of the seed (Plate XXV, fig. 6; Plate XXVI, fig. 4). As observed by us, the cotyledon first emerges through the micropyle as a knob-like protuberance. When seen under the microscope, the apex is seen a little projected with a very slightly raised circular ring having dots on its surface. The projected apex is the radicle which later on grows out as the root and the dots on the ring are the papillae from which numerous fine unicellular hairs grow out as a frill encircling the radicle (Plate XXVII, fig. 7). The same frill is also observed in the seedlings of *Monochoria*, a close relative of water-hyacinth. Different stages of the germination of the *Monochoria* seed are given in fig. 2. The function of these root hairs is very interesting. Arber [1920] says that in *Hippuris*, *Elatine hexandra* and many Helobieae such as *Zannichellia* the

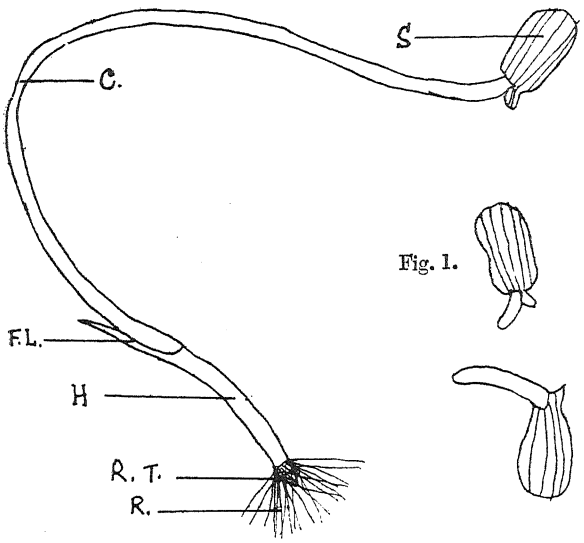


Fig. 4.

Fig. 1.

Fig. 2.

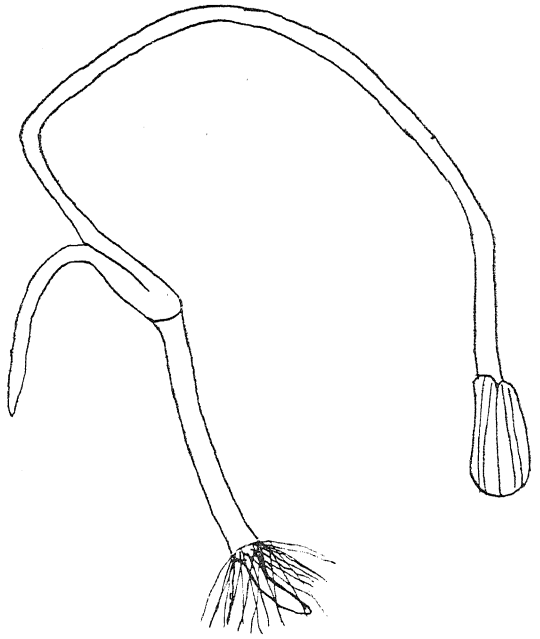


Fig. 5.

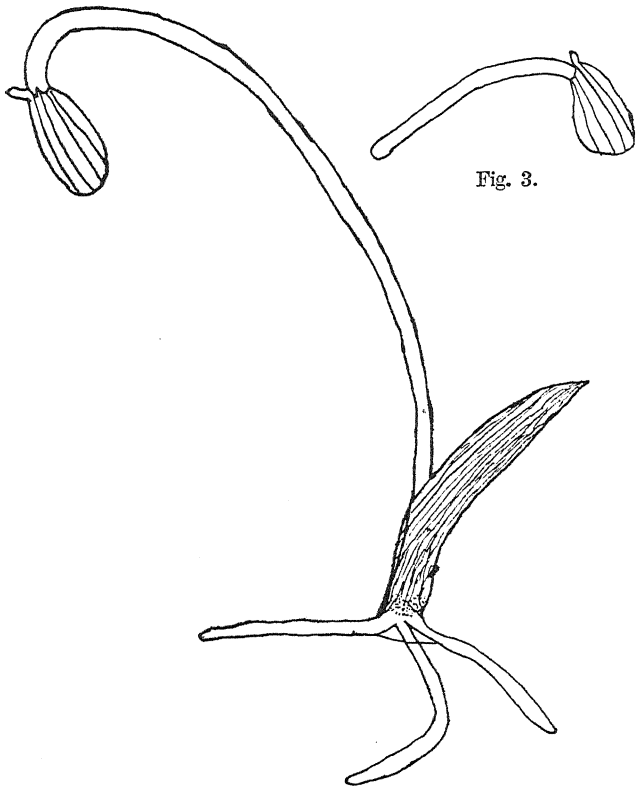


Fig. 3.

Fig. 6.



Fig. 8.

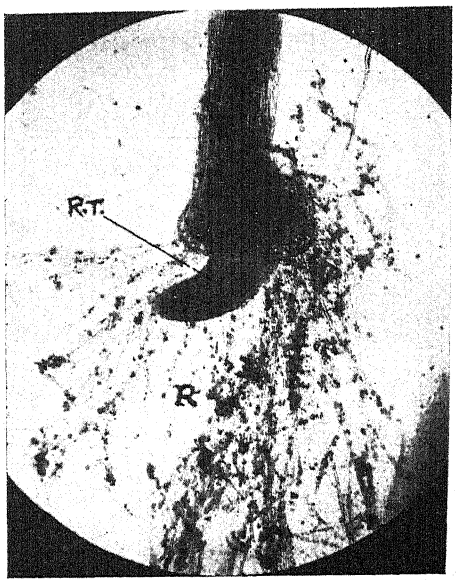
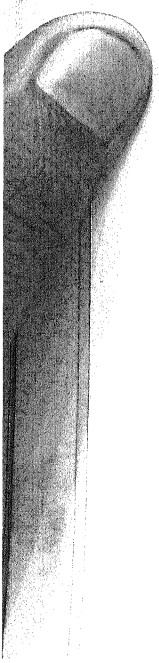


Fig. 7.

(*For explanation see page 429.*)



poor development of the radicle is often compensated for, at an early seedling stage, by the production of a tuft of very long root hairs, which grow out from the 'collet' or junction of hypocotyl and root. From field observations we have found that germinating seeds in all cases have been found on the surface, and germination takes place in the beginning of the rains or whenever the humidity, soil moisture and temperature are suitable. It is invariably found that a crop of seedlings appears after a shower of rain which seems, besides supplying the moisture, to expose the mud-covered seeds and thus give them access of oxygen. The function of the root hairs appears to prevent the young seedlings from being washed away and save the delicate radicle from being broken off. The root hairs anchor the cotyledon to the suitable soil and when it is safely fixed the radical tip grows and bores its way into the soil and establishes the plant. They might as well perhaps be serving as temporary absorbing organs for the plant so long as the radicle does not become active.

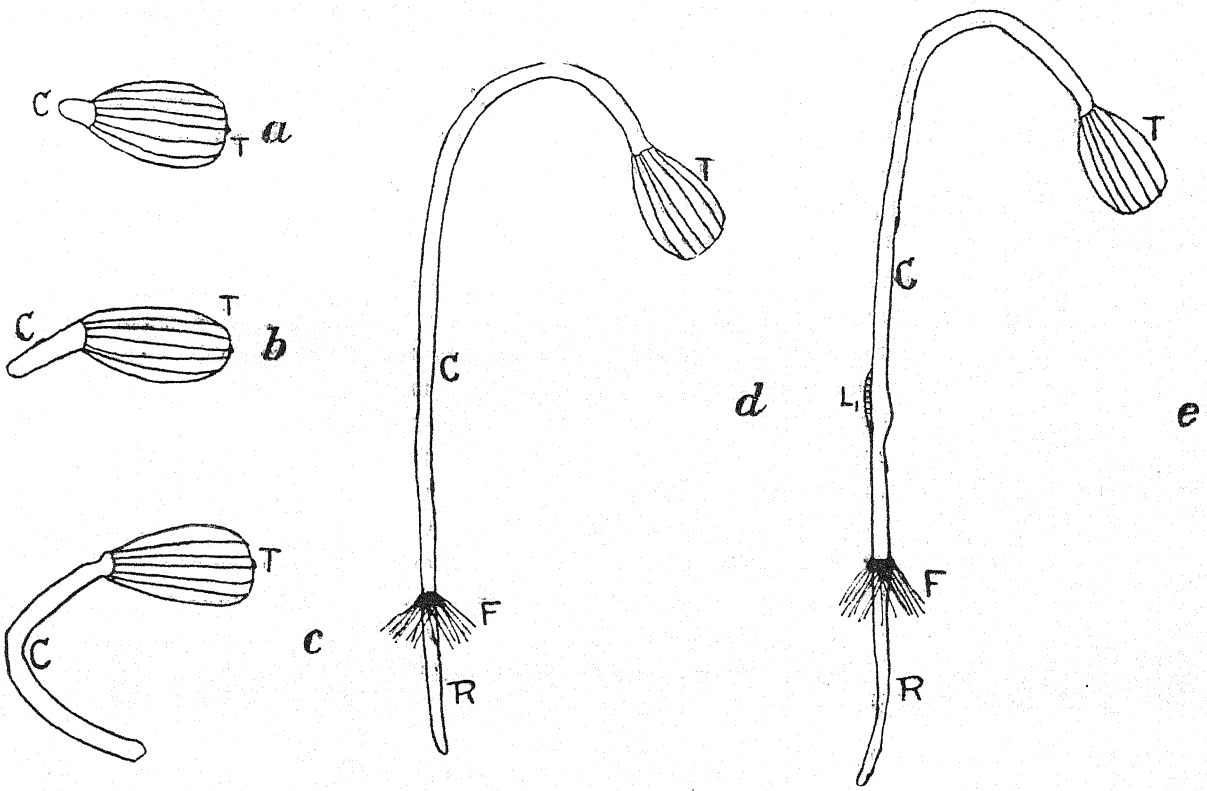


Fig. 2. *Monochoria hastæfolia*, showing various stages (*a-f*) in the germination of seed. T=Testa; C=Cotyledon; F=Frill of hairs; R=Root; L_1=First leaf.

The cotyledon grows out first, and if even after the limit of its length is reached, the radicle is not in suitable soil to grow, the hypocotyl is intercalated and the radicle is carried to a suitable depth and then its growth begins. During this time the cotyledon is green and probably supplements the plastic material in the seed. This explains why we find no hypocotyl in seedlings growing in very damp mud, because here the radicle is in suitable surroundings and soon becomes active. In some seeds germinated (in the laboratory) in water in a test tube we invariably found the presence of the hypocotyl in them (Plate XXVII, figs. 4, 5). The hypocotyl however, does not develop in seeds that germinated on soil or in those which were transferred to soil soon after the protrusion of the cotyledon (Plate XXVII, fig. 6).

Such intercalation in the axis takes place in other monocotyledons also such as rice. If the grains are sown at great depths, the coleoptile is carried up into the air by an intercalated piece of axis between the coleoptilar node and the scutellum while on the other hand, if the grain is sown on the surface, this intercalation does not develop.

What happens in *Eichhornia* is altogether different. If the seed germinates on the surface and the mud is suitably moist, then there is no hypocotyl, while if the water content falls below the required minimum, the hypocotyl is intercalated to carry the radicle down to proper depth.

The first leaf grows out when the radicle has become fixed in the soil. The tip of the cotyledon still remains in the seed.

Once the seedling is established, linear leaves come out in succession and form a mosaic. But there is no distinction between leaf-blade and petiole at this stage. It must here be noted that the leaves in mature *Eichhornia* appear dimorphic. The ligule of the older leaf covers the younger leaf as a membranous sheath and appears to be a different kind of leaf. In reality there is only one kind of leaf. In the leaves of a mature plant, the petiole swells into a "pseudo-bulb". There is no fixity of age when the "pseudo-bulb" appears, although the linear leaves of the seedlings produce aerenchyma (Plate XXVII, fig. 8). Seedlings grown in pots the soil of which was kept moist but not saturated, did not produce swollen petioles till they were four weeks old, while seedlings transplanted in earthen pots standing in water and thus having saturated mud, produce them in 14 days. One fact was clearly established that the seedlings require copious supply of free water in the soil for their healthy growth.

IV. Floating of water-hyacinth seedlings.

It has been noted before that the growth of seedling is affected by the amount of moisture. Now it has been observed that if water be lacking and the soil poor,

the leaves of the seedlings after a certain age spread horizontally, while if water is plentiful and the soil is rich, the leaves grow upwards. The first type of plant when submerged either does not float up at all or if it floats up at all, it takes a very long time to do so.

The other type in which the leaves grow upwards invariably floats up. The leaves execute a paddling movement, first moving upwards during 12 hours and then moving down. This coupled with the buoyancy of the leaves supplies the necessary energy for detaching the plant at the surface of cleavage already observed.

It was further observed that light hastens the floating. This is as should be expected, as light promotes photosynthesis and leads to accumulation of plastic material for growth and formation of meristem of the cleavage zone and also help the paddling movement which is no doubt a turgor movement. Boresch has observed by a series of experiments that the inflated form of petiole can be induced by full light, low temperature and free swimming life, whereas the converse conditions tend to be associated with the elongated form of petiole.

It has been noted before that floating always takes place in the evening between 5 and 7 o'clock. Apart from confirming this fact, it was found that there are two periods in the day when seedlings float up, namely between 7 and 11 in the morning and 4 and 8 in the evening. This is probably associated with the temperature.

V. Effect of hydrogen-ion concentration on the growth of water-hyacinth.

Hydrogen-ion concentration plays a very important role in the metabolism of plants. The physiological action of the free hydrogen-ions is considerable and a slight change in the hydrogen-ion concentration may have a critical effect. Water-hyacinth plant is amphibious in habit and grows where there is water. It was, therefore, thought that it might have a definite optimum range of pH value for its healthy growth. If this range could be found, then by changing the pH value of the water, it might be possible to check the growth and spread of water-hyacinth. Experiments were carried out to note the pH value of water where water-hyacinth was growing normally and also to see the effect of change of pH value on the growth of water-hyacinth.

All the experiments were done under conditions as nearly natural as possible. Big earthen pots were sunk into the ground in the open and plants were grown in them. Thus pH value could be controlled and the conditions approximated to the field conditions.

Our first endeavour was to see whether the water contained in the pots made up to a known pH value changes by itself without water-hyacinth plants growing in it. Two tubs were taken. To one an acid (sulphuric acid) was added to make the pH value up to 4·5 and to the other sodium hydroxide was added to make pH value up to 9·0. For twelve days it was kept under observation and pH value daily measured colorimetrically with ' Merck ' Universal Indicator. It was found that pH values both on alkaline and acid side became constant after a preliminary rise or fall.

When the above experiment was repeated with the water-hyacinth plant growing in water, it was found that pH values both from the acid and alkaline range gradually changed towards the neutral value and after some time remained within a range between 6·0 and 8·0.

Thus water-hyacinth like most plants is able to flourish within a definite range of pH values, namely 6·0-8·0. Having thus determined the range of pH value for growth of water-hyacinth, experiments were arranged for measuring growth in pH values on either side of this range.

Seven tubs of the same size and depth were taken. All of them were filled with tap water. One was kept as a control without acid or alkali being added to it (pH 7·0-7·5). In the other tubs requisite volumes of sulphuric acid and sodium-hydroxide solutions were added to have pH values ranging from 4·0-5·0 ; 5·0-6·0 ; 6·0-7·0 ; 7·0-8·0 ; 8·0-9·0 ; 9·0-10·0. Plants of nearly the same age and development were allowed to grow in the respective tubs. Equal number of plants, with equal number of leaves, were taken in each case and the pH value was measured daily, care being taken to keep the pH value constant.

Growth rate was measured in terms of volume on each fourth day, the measurement being carried on up to the 36th day. In measuring growth rate, we took the volume of the plant as our basis. This gives a fairly accurate idea of the growth rate, as in the case of the water-hyacinth, the leaves persist for a long time and they certainly did persist during the period of experimentation. The volume was measured by taking out the plants from the tubs, and keeping them slantingly (from 5-10 minutes only to avoid drying up) on a rack so that superfluous water might drain off. They were next kept in a sufficiently broad graduated cylinder. Then by placing a mica sheet on the top of the plants they were pushed down and were dipped into the water by means of a dissecting needle. The same mica sheet and the needle were always used till the end of the experiment, so that the volume of the sheet and the needle could be neglected. In this way we could find the total growth of the plants including the growth of the roots, expansion of the bulbs and the growth of the leaves. The percentage growth rate was calculated and the results are represented in the table below.

Percentage growth of water-hyacinth in different concentrations of hydrogen-ion.

pH	4·0-5·0	5·0-6·0	6·0-7·0	Control	7·0-8·0	8·0-9·0	9·0-10·0
1st day . .	100	100	100	100	100	100	100
4th day . .	133	128	150	137	123	119	110
8th day . .	133	143	170	181	154	154	116
12th day . .	150	157	200	243	200	173	138
16th day . .	172	180	300	337	281	196	144
20th day . .	180	183	320	387	300	208	150
24th day . .	183	196	390	468	309	215	155
28th day . .	183	212	460	500	381	238	155
32nd day . .	183	236	525	550	400	250	155
36th day . .	183	242	580	600	445	269	155

From Fig. 3 where the above results are plotted we can see that growth rate is highest in the control. Then comes pH 6·0 and pH 8·0. In other ranges of pH, growth rate is comparatively very much checked, so much so that at both the extremes pH 9·0-10·0 and 4·0-5·0 after a time the growth is totally checked. If we take the growth rate on a particular day, in different values and plot them together then we get a curve shown in Fig. 4. Olsen as quoted in Lundegardh [1931] following a statistical method in determining the relation between distribution of plants and hydrogen-ion concentration found that every species has got an optimum range of hydrogen-ion concentration. His curve of frequency of a number of species closely resembles our curve of growth rate in the water-hyacinth.

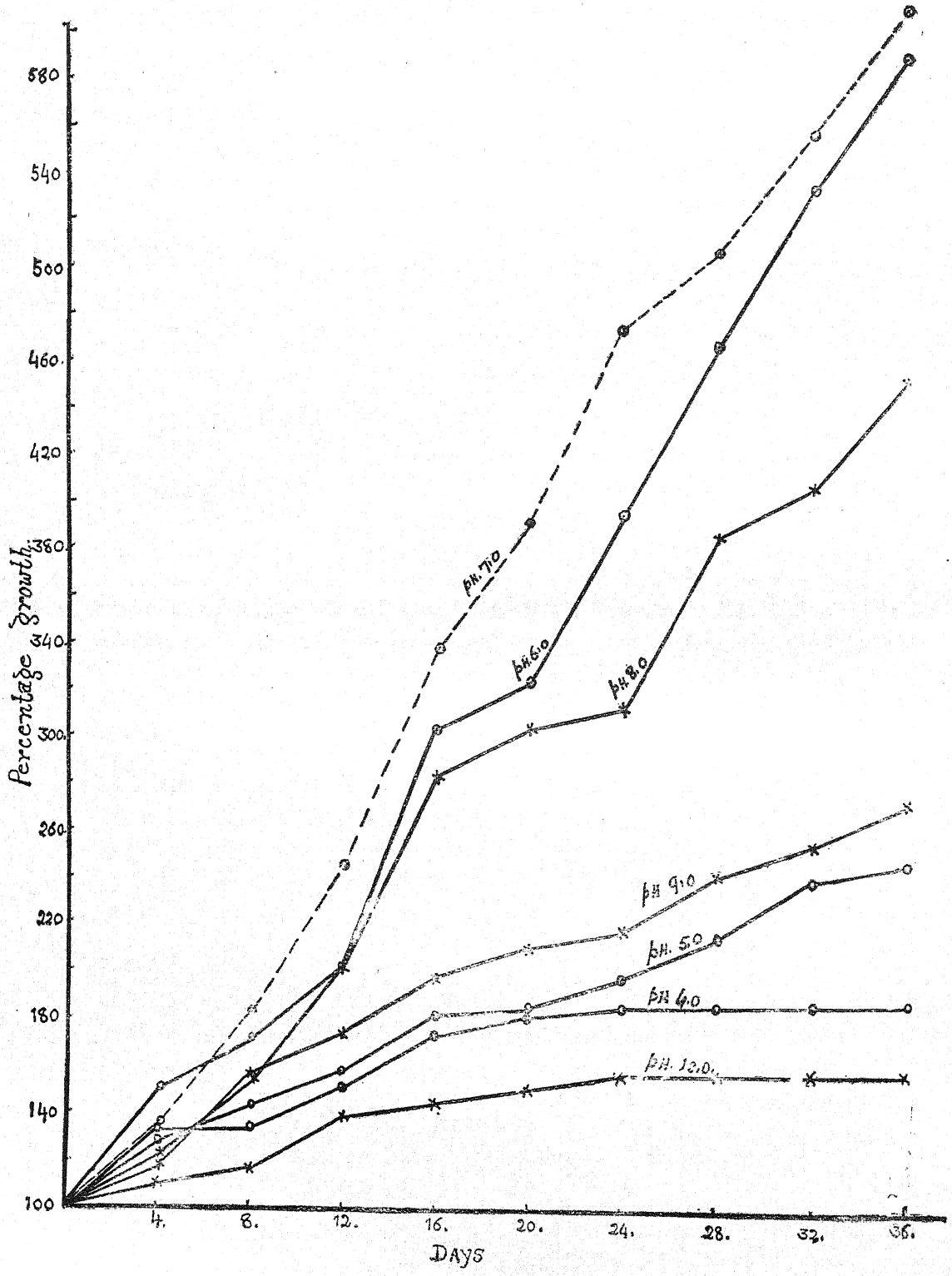


Fig. 3. Growth rate of water-hyacinth in different hydrogen-ion concentration.

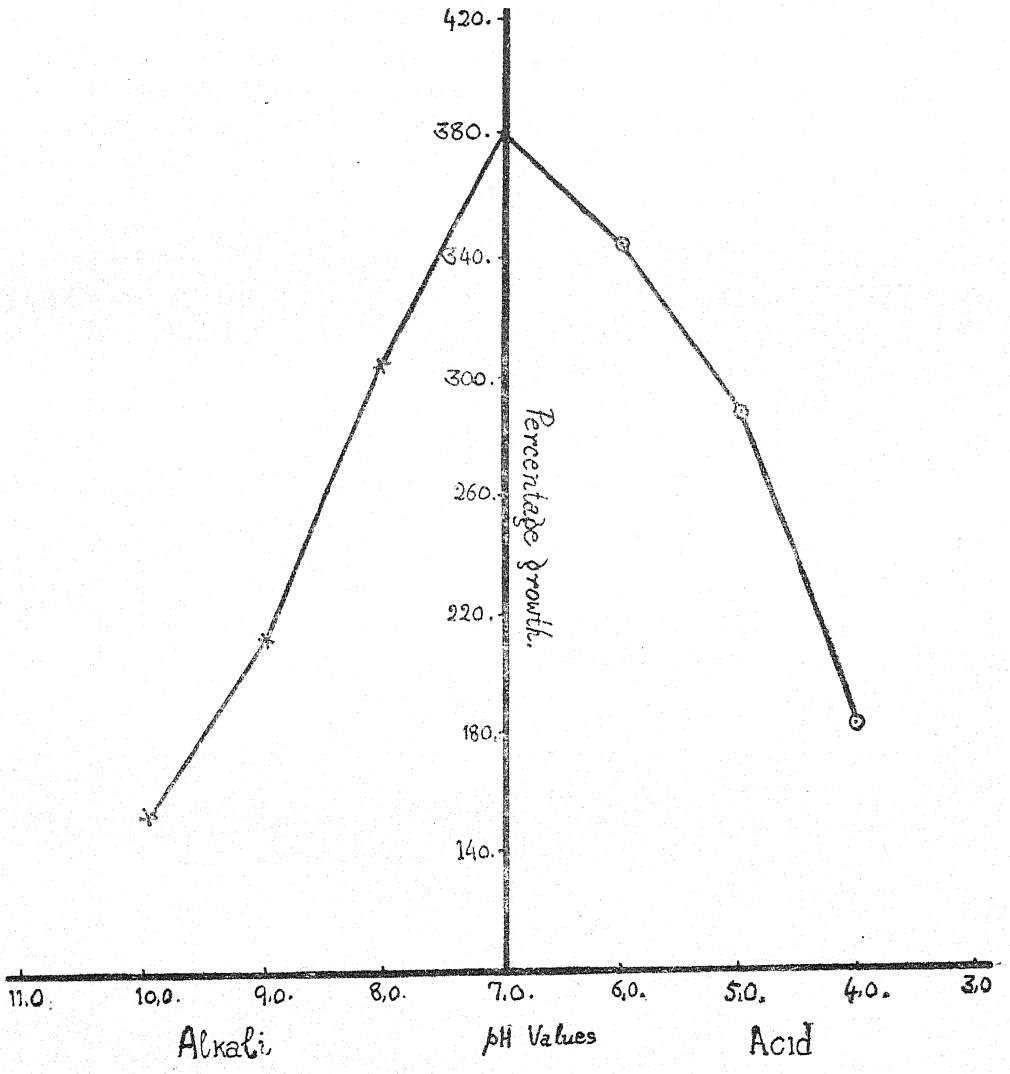


Fig. 4. Graph derived from Fig. 3 at the points corresponding to 380 and 20 showing the pH value in which water-hyacinth flourishes.

Our experiments conceived without the knowledge of Olsen's work show that the distribution of water-hyacinth could be checked by altering the hydrogen-ion concentration of the medium but it is doubtful how far this may be practicable. When one has to deal with large stretches of water, it is difficult to change the hydrogen-ion concentration. There is an optimum at 7·0 and a fall on both the sides. The results thus showed that water-hyacinth, like most plants, was

able to flourish within a considerable range of pH values, though a definite optimum could be distinguished.

The question naturally arises how the plants and especially the roots which always remain suspended in water adapt themselves to such extremes of pH values as 4·0-5·0 and 9·0-10·0. Though the growth rate is totally checked, yet the plants survive. The roots which are liable to be more affected we find that instead of soft and flexible roots, they have become hard and somewhat brittle. Instead of fine laterals covering throughout the length of the root all of them have been shed except a frill near the tip.

Although the growth of water-hyacinth was checked by changing the hydrogen-ion concentration beyond the tolerable range on either side, and some plants died, yet many plants continued to live. This we think is due to the antagonistic action of calcium present in the water-hyacinth plant. Many workers have shown that calcium does antagonise the effect of hydrogen-ion concentration. In the case of water-hyacinth, the cells are rich in calcium oxalate crystals. These must be escaping from decayed leaves and, falling into the medium, probably antagonise the effect of hydrogen-ion concentration.

We shall see later in another connection, the antagonising action of calcium and potassium.

Summary.

(1) The water whose pH value was made to 9·0 keeps constant without water-hyacinth growing in it; but with water-hyacinth tends towards 7.0 and keeps constant at pH 8·0.

(2) The water whose pH value was made to 4·0 keeps constant without water-hyacinth growing in it, but with water-hyacinth tends towards the normal and keeps constant at pH 6·0.

(3) Like most plants water-hyacinth is able to flourish in a very wide range of pH values though a definite optimum could be distinguished.

(4) Growth rate on either side of the optimum range of pH value is checked, but the plants are not killed.

(5) Calcium present in the leaves as raphides, antagonises the hydrogen-ion concentration, however small the quantities may be, by escaping from the dead leaves which decay in the water.

VI. Drought resistance in the water-hyacinth.

Water-hyacinth plant is capable of growing as a water-aswell as a land-plant. It is a common field observation that water-hyacinth survives in tanks from which all the water has dried up, and the soil is just moist to support plant life. Therefore, it was thought desirable to study how the plant adapts itself to this environ-

ment to tide over the period of drought during the summer season. With this end in view experiments were planned and performed to find out :—

(i) the relation of water content of the soil to the survival of water-hyacinth

(ii) the change in the osmotic pressure in the cells of water-hyacinth when it migrates from water to land and is subjected to gradual desiccation

and

(iii) the root development as water content of the soil decreases.

(*i*) *The relation of water content of the soil to the survival of water-hyacinth.*

The relation of water content of the soil to the survival of water-hyacinth was found by allowing equal number of plants of the same age and growth to grow in earthen tubs of the same size. The same quantity of soil with similar texture and water holding capacity was used. The plants were allowed to grow in the tubs for twenty to thirty days so that the plants might adapt themselves fully and develop land roots. They were kept in the green house and irrigated daily with equal quantities of water. When they had fully established themselves into the ground, the tubs were brought out from the green house and kept under the sun. The experiments were done in the month of April when both the soil and the atmosphere were dry. The initial water content of the soil was found by boring out a certain weight, of the soil from a definite depth from two or three places and allowing it to dry up in a hot air-oven at 80° C. The average value was taken. This gives the absolute water content of the soil. Then the plants were kept under conditions of drought, *i.e.*, the daily water supply was cut off. A number of pots with water-hyacinth growing in them were experimented upon and arranged in series. After every three days of drought three pots were watered and water content of the soil and the osmotic strength of the cells of the stem were noted just before watering them. The number of plants surviving after watering were also noted as shown in the Table I.

TABLE I.

Showing relation of water content of the soil to the survival of water-hyacinth.

Days of drought	Water content absolute in per cent. soil	Relative water content in per cent. saturation value	Per cent. survival
3	12·7	39·9	100
6	9·4	29·5	100
9	6·6	20·75	62
12	1·8	5·7	25

It is, therefore, seen that though the water-hyacinth is an aquatic plant, yet, when grown as a land plant, it can survive in very low water content of the soil—5·7 per cent. of the saturation value. The effect of drought is first felt by the transpiring leaves which begin to wilt from the farthest end of the veins or veinlets. Hence it was thought desirable to find out the water content of the soil when the tips of the leaves begin to wilt. This would give the ' wilting co-efficient of the soil' which, according to Briggs and Shantz, means the moisture content of a soil expressed as a percentage of its dry weight, at a time when the leaves of a plant growing in this soil show the first signs of permanent wilting. This will give the minimum quantity of soil moisture available for the roots, from which absorption by the roots could take place. The average content of the soil when wilting sets in, in case of water-hyacinth is 80 per cent. of the dry weight. In terms of relative water content in percentage saturation, we find that the water-hyacinth plant shows signs of permanent wilting at 25·35 per cent. (Table II) of the saturation value. But even when the plants were subjected to drought amounting to 5·7 per cent. (Table I) of the saturation value, 25 per cent. of them were found to survive. It therefore shews that although the effect of drought is very early felt by the water-hyacinth plant, it has a great capacity of resistance. This is further corroborated by the fact that osmotic strength of the cells of the stem increases to a great extent.

TABLE II.

Percentage of water in the soil when water-hyacinth shows signs of permanent willing.

No. of observations	Wt. of the soil in grms.	Wt. of the air-dried soil (kept in the sun) in grms.	Water content in grms.	Percentage water content	Relative water content in per cent. saturation value
1	16·59	15·425	1·165	7·3	22·9
2	12·683	11·515	1·168	9·2	28·9
3	12·622	11·8	0·822	6·5	20·4
4	10·46	9·484	0·976	9·3	29·2
			Average .	8·0	25·35

(ii) Change in the osmotic pressure of the cells of water-hyacinth.

The osmotic concentration of the cells of the stem at different periods of drought was measured by contraction and expansion method, as the plasmolytic

method was found unsuitable. By means of a sharp cork-borer, the core of the stem with portions of cortex all round it, was bored out in order to have equal diameter of discs of tissue in all the experiments. From this cylindrical core sections of equal thickness were cut off and dipped in graded molar solutions. They were then measured by means of a fine divider, and plotted against concentration of the solution on a graph. By extrapolation where the curve cuts the abscissa-axis the osmotic strength was found. When the values of osmotic strength were plotted against the number of days of drought we get a curve rising gradually (Fig. 5). This increase in the osmotic strength explains how the plant is able to absorb an adequate supply of water from the dry soil.

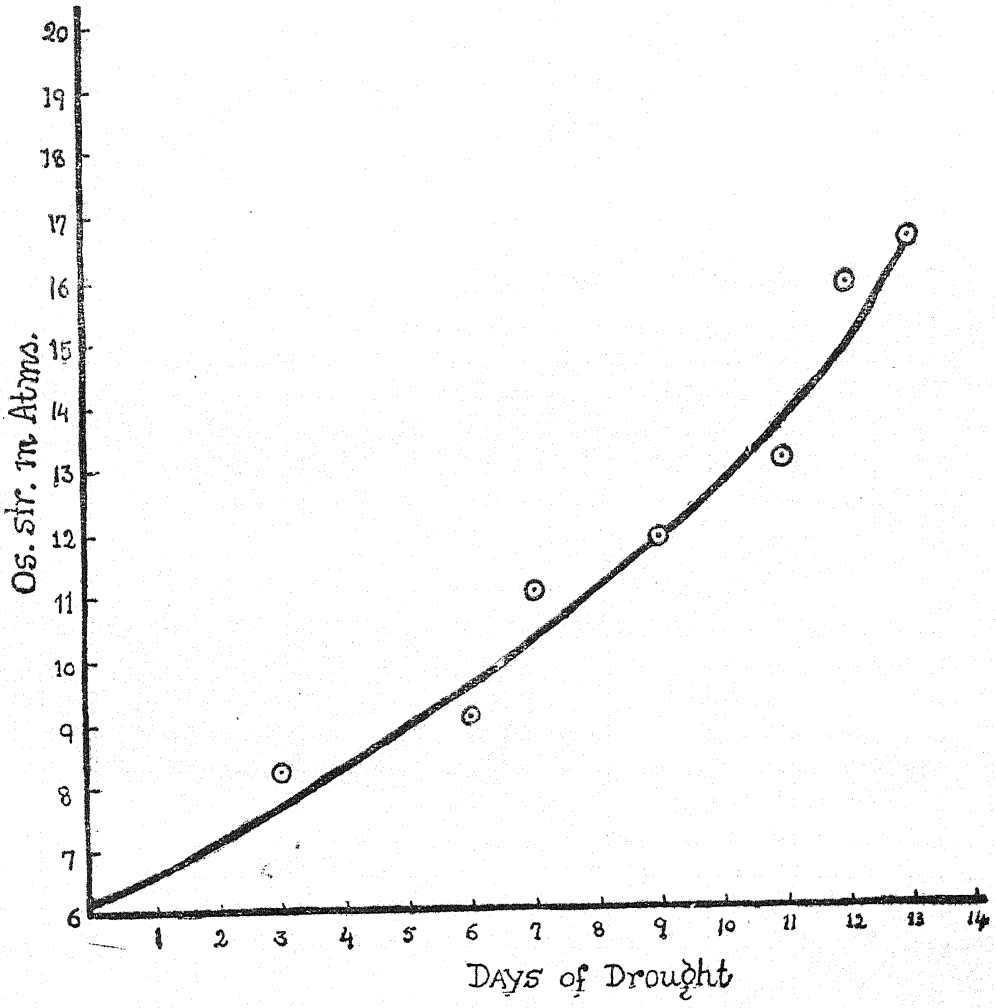


Fig. 5. Drought resistance: graph showing the change of osmotic pressure with drought.

(iii) Root development as water content of the soil decreases.

Coupled with high osmotic strength the root development and the transpiring surface also are affected. The total length of the roots in the floating forms were found out. The root washing *in situ* was done in plants growing as land plants and under drought to find out the depth of the roots and the total length. In order to compare the root development with the transpiring surface, the total area of the leaves was found by tracing the outline of the leaves on paper and measuring the area by means of a planimeter. It was found that the ratio of the total leaf area to that of the total length of the roots decreases from 5·6 to 2·5 under the conditions of drought.

The stem portion of the water-hyacinth plant, which is protected by leaf bases, resists the drought to a great extent on account of the presence of mucilage at the axils of the leaves. Life persists in the inner cone of the stem for a considerable period under drought, when to all outward appearance it seems dead. Slight traces of moisture would revive it to sprouting again.

Summary.

Water-hyacinth is capable of growing as a land plant and resists a considerable degree of drought. It was found that—

(*a*) Water-hyacinth can suruive in a very low water content of the soil *i.e.*, 5·7 per cent. of the saturation value.

(*b*) The osmotic strength of the cells in the stem of water-hyacinth increases as it migrates from water to land and is subjected to gradual desiccation. From about 6 atms. in floating plants it rises to about 17 atms. under drought.

(*c*) The ratio of the total leaf area to that of the total length of the roots decreases from 5·6 to 2·5.

VII. Effect of poisons on water-hyacinth.

This is not the first time that the effect of poisons on the water-hyacinth has been studied. Various authors have tried various poisons on the plant. MacLean [1922] tried a number of such poisons. Our object was to reinvestigate this problem. With this end in view, water-hyacinth was grown in pots and solutions of inorganic poisons were added to the water. Of all the poisons tried, copper sulphate and barium chloride seem to produce the greatest effect.

On account of the cheapness of copper sulphate, a detailed investigation was undertaken of this salt. Solutions of various strengths were made up and water-hyacinth plants were put in them and effect noted. Various concentrations were tried on the water-hyacinth and *Pistia*, ranging from 0·006 per cent. to 0·024 per

cent. It was found that a concentration of 0·018 per cent. or more killed the water-hyacinth while *Pistia* was killed even with a concentration of 0·006 per cent.

It was noted that the lower concentrations were not so effective as the higher ones, and repeated applications were necessary to produce lethal effect, while in comparatively stronger solutions one dose was sufficient to produce the effect.

It was further noticed that when plants had been grown in sub-lethal concentrations for some time, they could tolerate higher concentrations better than plants which were put in these solutions for the first time. Thus the plants which had been growing for 27 days in 0·006 per cent. solution of copper sulphate, were not killed even if the concentration was raised to 0·018 per cent. through 0·012 per cent. while plants grown in 0·018 per cent. copper sulphate directly die off.

Another interesting observation was that although most plants died off, occasionally one found in one or two pots a few plants surviving the application of copper sulphate. It was suspected that this survival might be due to antagonism of calcium. The water-hyacinth plant has a large number of raphides in its cells, which are crystals of calcium oxalate. If one or two leaves decayed, they would liberate these crystals into the water and it was thought possible that the calcium was antagonising the action of copper and hence some plants were found surviving the application of copper sulphate. This suspicion was confirmed by subsequent experiments as described below.

Mr. B. N. Sinha conducted a set of experiments in which water-hyacinth plants were floated in glass jars containing 3,000 c.c. of water to which had been added copper sulphate and various calcium salts as noted in the following table. There was a control in water alone.

TABLE.

Water-hyacinth plant No.	Chemicals mixed with 3,000 c. c. of 0·05 per cent. solution of $CuSO_4$
1	None (control).
2	1·0 grm. calcium nitrate.
3	1·0 „ „ chloride.
4	1·0 „ „ carbonate.
5	1·0 „ „ phosphate.
6	1·0 „ „ fluoride.
7	1·0 „ „ oxalate.
8	0·75 „ „ „
9	0·5 „ „ „
10	0·25 „ „ „

After 24 hours it was observed that No. 5, *i.e.*, the plant which received copper sulphate and calcium phosphate had suffered least and the plant No. 8, *i.e.*, the one which received 0·05 per cent. of copper sulphate and 0·025 per cent. of calcium oxalate was the next best. Even after 48 hours No. 5 continued to be the best. This indicates that calcium phosphate antagonises copper sulphate to a greater extent than calcium oxalate. On the third day, however, the plant receiving calcium oxalate showed signs of poisoning as indicated by wilting and browning of leaves. This investigation is being continued further.

Observations recorded here seem to explain why spraying experiments have failed on a field scale. The reasons may be two-fold. Firstly, lethal concentration of the spraying fluid might not be reached, and secondly, various antagonising salts might be present in the water which would nullify the effects of poisons applied.

While experimenting with copper sulphate it was noticed that the leaves showed signs of wilting and browning at the edges. That meant that either the absorption of water was adversely effected by the application of copper sulphate or the transpiration was favoured, in either case producing a negative water balance. In order to determine this, experiments were planned and Mr. S. M. Sircar carried them out to determine the effect of various concentrations of copper sulphate on the absorption and transpiration of water-hyacinth. The apparatus used was a simple one as shown in the photograph (Plate XXVIII). Absorption was measured by the fall in level in the narrow limb and the transpiration by loss of weight of the whole system. The results are shown in the adjoining graph (Fig. 6). Although the question is still under investigation, it is clear that absorption has a tendency to lag behind transpiration. That the roots of water plants are affected by copper sulphate is corroborated from the study of the effect of copper sulphate on *Pistia*. It will be described later that the roots fall off on treatment with copper sulphate.

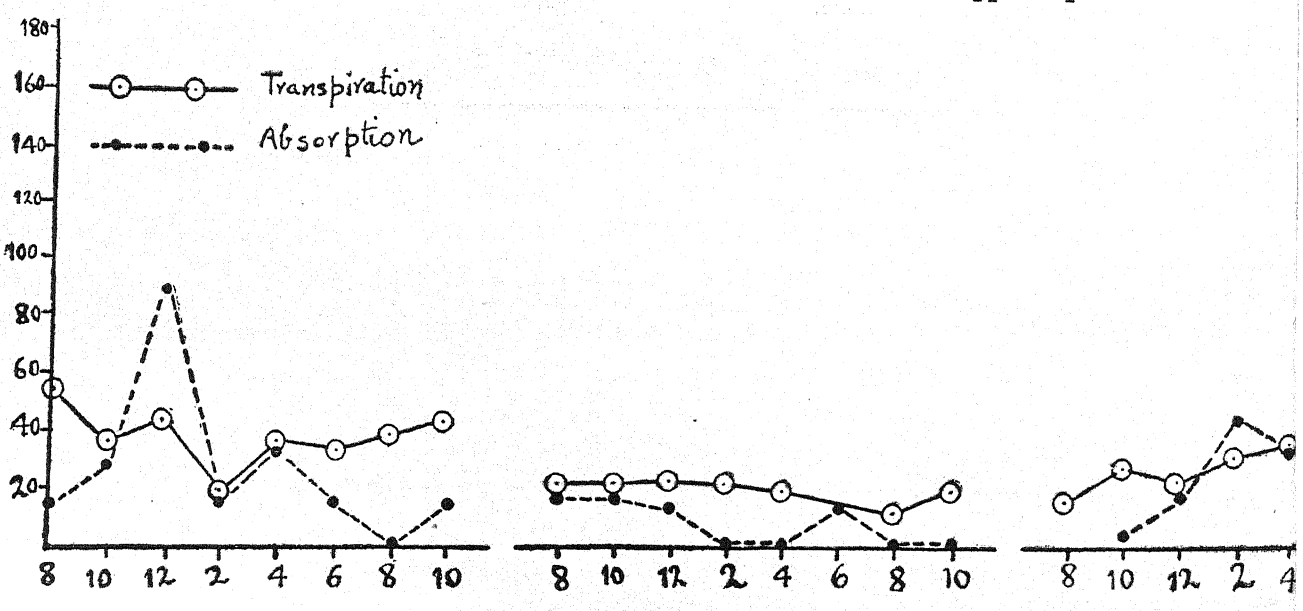
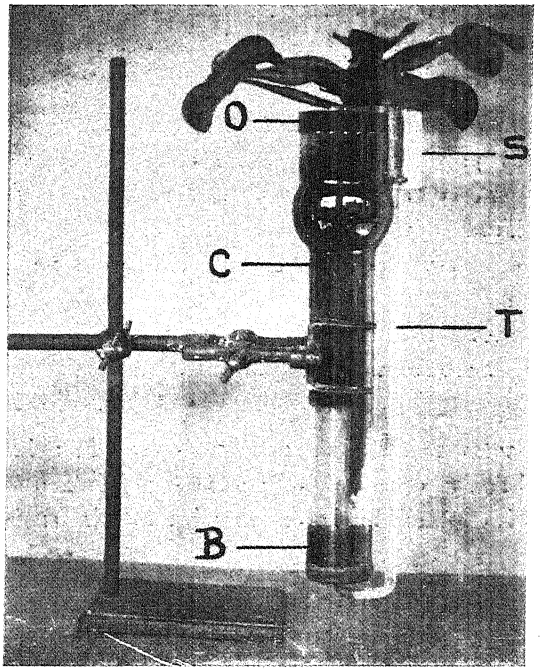


Fig. 6. Effect of 0·1 per cent. copper sulphate on transpiration and absorption of water-hyacinth.



Apparatus to determine absorption and transpiration in water-hyacinth plant
B=Rubber bung; T=Glass tube; S=Scale-index;
C=Glass chimney; O=A layer of oil.

Summary.

Copper sulphate was found to be the most effective poison against water-hyacinth, the minimum lethal dose being 0·018 per cent.

It was observed and experimentally verified that calcium salts antagonise copper sulphate, calcium phosphate being more effective than calcium oxalate.

A preliminary study on the effect of copper sulphate, on the absorption and transpiration was made. It was found that on the application of copper sulphate the absorption lags behind transpiration. Further work on this is in hand.

VIII. Relative growth rate of water-hyacinth and *Pistia*.

It is a common field observation that tanks, which are cleared of water-hyacinth were soon filled with another pest, *Pistia stratiotes*. This fact was also reported to us by the district authorities who were in charge of cleaning the tanks. This led us to study the relative growth rate of the two pests.

In two tubs of area 353 sq. in. each, fourteen *Pistia* plants and seven water-hyacinth plants were allowed to grow separately. The growth rate was measured in terms of their volumes, and percentage growth rate calculated on the initial value.

No. of days	Percentage growth rate	
	Water-hyacinth	*Pistia*
1st	100	100
4th	20	25
8th	30	40
12th	80	64
16th	100	75
20th	200	125
24th	220	200
28th	260	225
32nd	290	290
36th	320	350
40th	370	550
44th	480	962
48th	680	1,150

From the graph (Fig. 7) it is seen that water hyacinth doubles itself on the 16th day while *Pistia* doubles itself on the 18th day. Obviously growth rate of water-hyacinth is more rapid than that of *Pistia*. Later on, the growth curve of *Pistia* rises more than that of water-hyacinth which is due to the production of a large number of plantlets by the mother plants in case of *Pistia*. Within 40 days *Pistia* plants have filled totally an area of 353 sq. inches and have produced 240 plants, while water-hyacinth could not fill the area and produced only 11 plants.

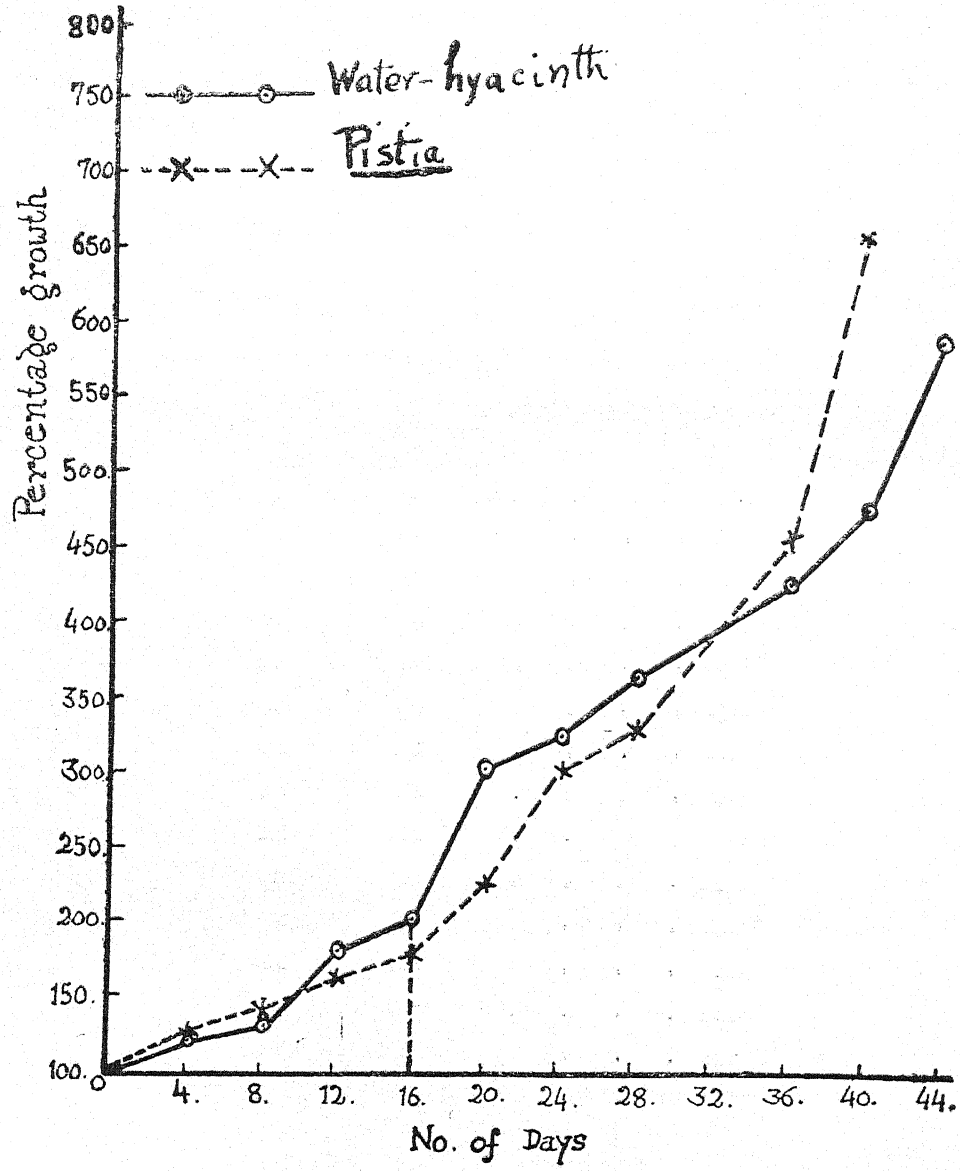


Fig. 7. Comparative growth rate of water-hyacinth and *Pistia*.

Pistia plants when fully developed do not reach more than six inches in height and perhaps the limited growth on this side is utilised in spreading. So when *Pistia* is allowed to grow separately, it spreads more quickly than water-hyacinth; but in nature water-hyacinth kills the *Pistia* plants by its luxuriant vegetable growth.

IX. Other water weeds.

Monochoria.

Of the weeds other than water-hyacinth, *Monochoria hastæfolia*, *Pistia stratiotes* and *Salvinia cucullata* deserve notice. *Monochoria hastæfolia* is a member of the family Pontederiaceæ to which the water-hyacinth belongs. It is rather common and as Peck [1930] mentions in his book already referred to, it is sometimes mistaken for water-hyacinth. But as the plant is indigenous, it is not so troublesome nor does it spread so much as its cousin—the water-hyacinth. We had the opportunity to study only the germination of seeds which agrees in all essentials with that of water-hyacinth. Fig. 2 depicts seedlings in various stages of germination and shows the resemblance to the water-hyacinth in a remarkable degree.

Pistia.

(*i*) *Growth.*—This weed is also very common in ponds and other stretches of water in these parts. It is indigenous but it was ousted from many stretches of water by the water-hyacinth. When, however, the water-hyacinth was cleared, the tanks, etc., were soon filled with this weed. The spreading is so rapid that it caused suspicion in the minds of the executive officers who were engaged in clearing, as to the utility of the operations. We measured the relative rate of spreading of the water-hyacinth and *Pistia*. The details have been given on page 421. It is found that although *Pistia* spreads more rapidly than the water-hyacinth, being a dwarf floating plant, it does not cut off light from the water below so completely, as a dense growth of water-hyacinth does.

(*ii*) *Seeds and germination.*—While experimenting with *Pistia*, it was thought desirable to find out the mode of propagation and perennation in this plant. Although this plant spreads, like the water-hyacinth, mostly by vegetative means, it sets seeds quite freely in the cold weather (November and onwards). The seeds are heavier than water and when mature, they are discharged from the capsule and sink under water. Next spring or summer, when favourable conditions appear, the seeds germinate. The first stage of germination consists in the protrusion of a white spongy structure (cotyledon) through the micropyle. As soon as this reaches a certain stage (Fig. 8) the seed floats up and later on, the hair-covered leaves come out and the plant establishes itself as a floating organism by producing roots. The germination of the seeds of this species agrees with that described by Engler [1920]

after Klotzsch and Hegelmaier for *P. Turpini*. The interesting point here is the floating of seedings. This is altogether different from what has been described for the water-hyacinth.

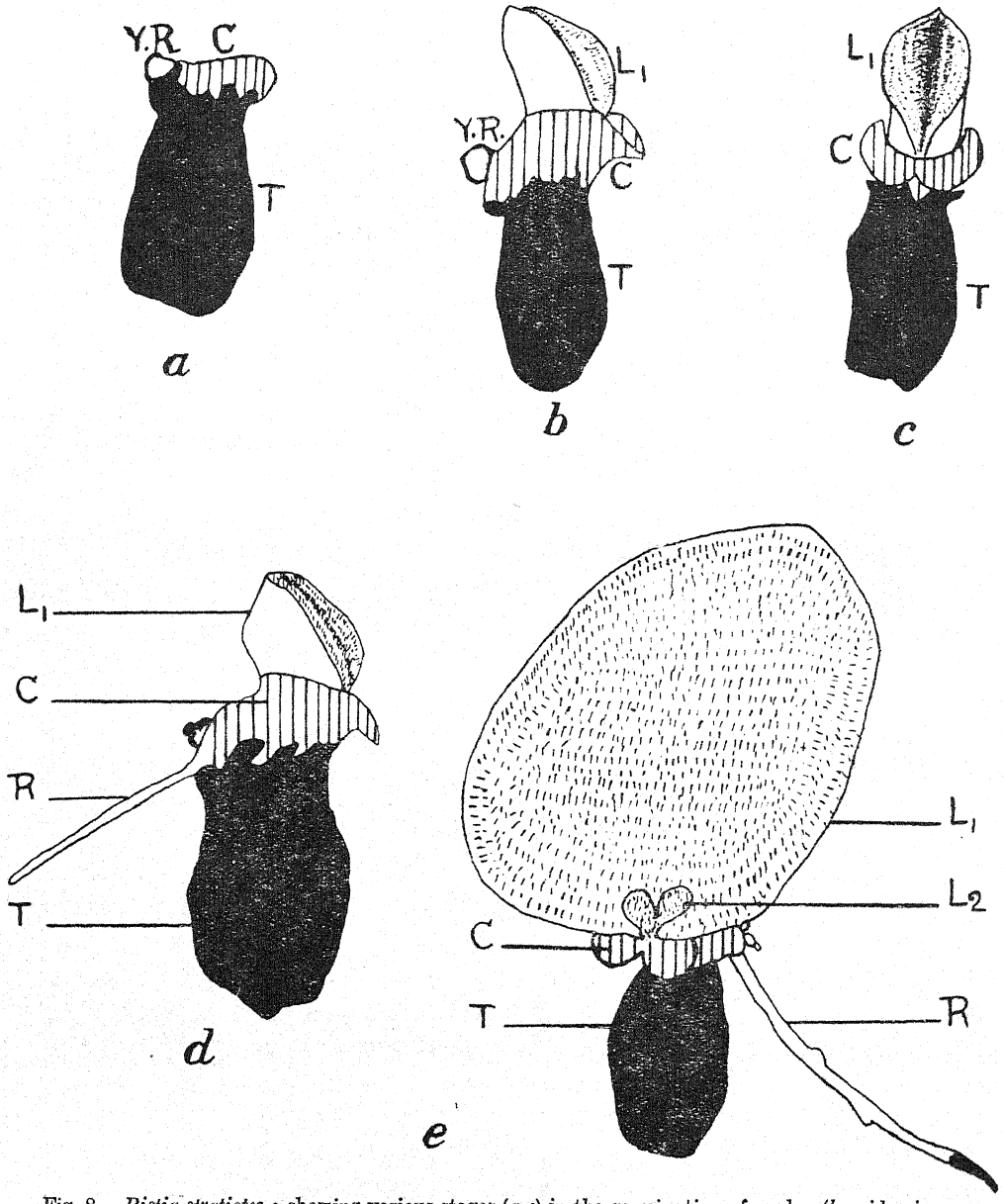


Fig. 8. *Pistia stratiotes* : showing various stages (*a-e*) in the germination of seed. (*b*=side view; *c*=front view of *b*). L_1=First leaf; L_2=Second leaf; C=Cotyledon; R=Adventitous root; YR=Young adventitous; T=Testa.

PLATE XXIX.

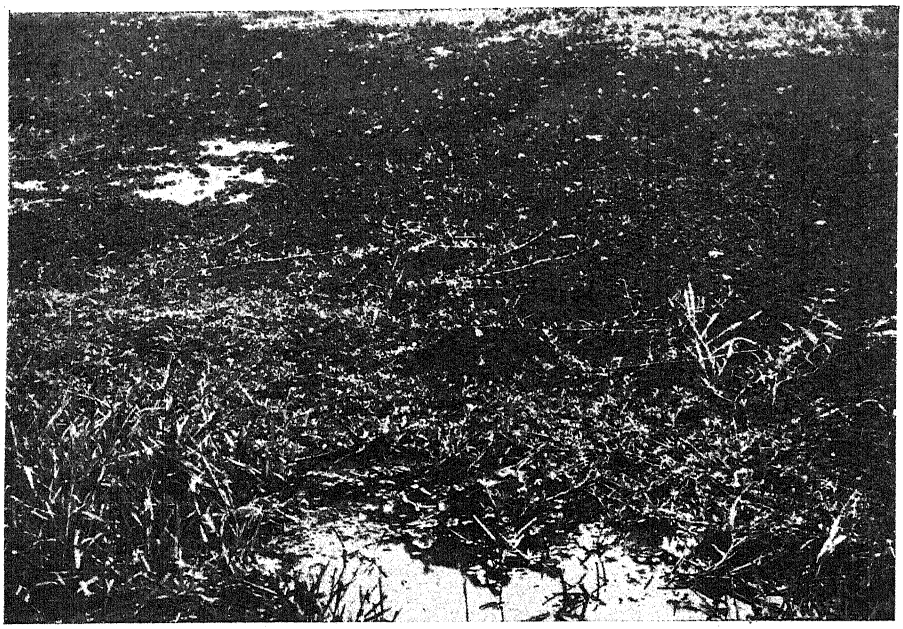


Fig. 1. *Salvinia cucullata :* Showing luxuriance of vegetation in the control area.

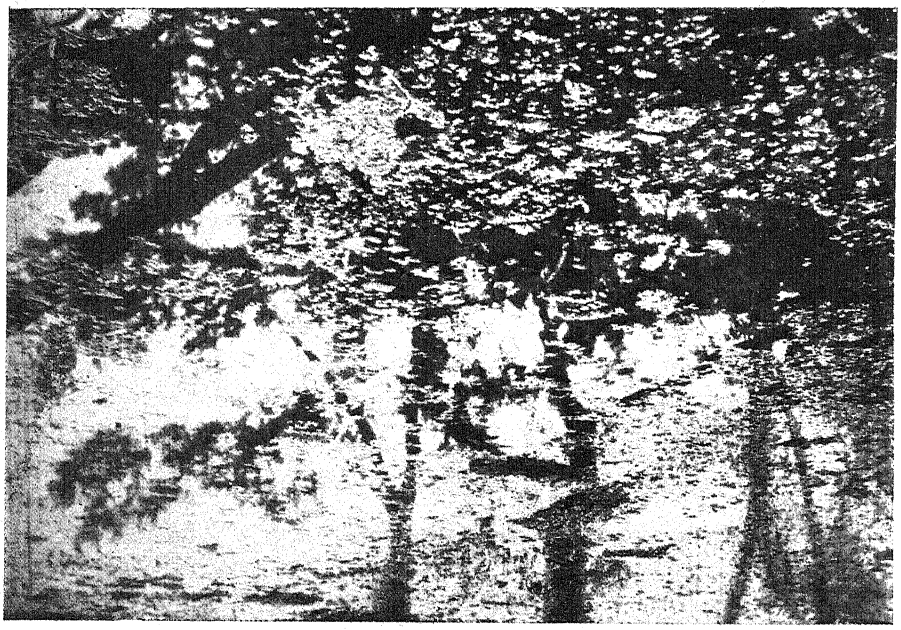


Fig. 2. Dead plants of *Salvinia cucullata* floating about in the experimental area sprayed with kerosene. The cleared stretch of water reflects the trees in the background.

As the seeds are fairly small and when the seedling floats, the testa is almost completely under water and the cotyledonary rim seems to support the seedling in its floating. It was thought that surface-tension might be playing an important part. If the assumption was correct, then kerosene oil or any other substance which would reduce the surface-tension would tend to make the seedling sink. With this idea, when kerosene was applied to tubs containing seedlings and adult plants, not only the seedlings sank, but the adult plants also suffered and ultimately died.

Effect of poisons on Pistia *and* Salvinia.

(*i*) *Kerosene.*—This led us to investigate the effect of kerosene on adult *Pistia* plants and also on *Salvinia* which also grows in close formation on the surface of tanks and pools. Both *Pistia* and *Salvinia* have one common feature, namely, the leaves are covered with hairs. The hairs help the plants in floating up if submerged, as they entangle a comparatively large quantity of air which increases the buoyancy of plants. They also prevent the wilting of the leaf-surfaces.

When *Pistia* or *Salvinia*-formations are sprayed with kerosene its first effect is to wet the surface of the leaf and thereby cause the plants to sink under water.

Whatever may be the way in which kerosene works, the effect is that both *Pistia* and *Salvinia* die off if they come under the kerosene spray. The two photographs, one of the control area (Plate XXIX, fig. 1) and the other of the experimental (Plate XXIX, fig. 2), show the effect of kerosene on *Salvinia*. It will be seen that in the experimental area *Salvinia* has almost disappeared and only dead plants are floating, although before spraying the formation was as dense as the control area. Three sprays are most effective. The quantity of kerosene is 4 litres on an area of 400 sq. ft.

(*ii*) *Copper sulphate.*—While experimenting on the effect of copper sulphate on water-hyacinth, that poison was tried on *Pistia* as well. While the water-hyacinth requires 0·018 per cent., *Pistia* requires 0·012 per cent. only for lethal effect. Even 0·006 per cent. kills *Pistia*. Leaves show signs of wilting within 16 hours. In 48 hours roots fall of the plants (Plate XXX, fig. 2 *cf.* fig. 1). The rosette of leaves then disintegrates and the plant dies. If the plants are transferred to fresh water after the roots have fallen off, they do not survive.

Salvinia is killed by the same concentrations as *Pistia*. The absorbing organ, which is morphologically a leaf, does not fall off nor do the leaves disintegrate immediately. They merely become black.

It may be mentioned here that the lethal concentration of copper sulphate is also lethal to fish.

X. General summary.

Water-hyacinth.

The seeds of water-hyacinth remain dormant at least for one season, *i.e.*, from November to June, and retain their viability for serveral years.

The embryo is fully developed before the capsule bursts.

The dormancy is caused by the hard seed-coat which offers mechanical resistance and prevents the entry of oxygen into the seed. When the seed-coat gets loosened either by alternate wilting and drying or by chemical action, germination is facilitated by the entry of oxygen.

Stages in the germination are described.

The mechanism of the floating of submerged water-hyacinth seedlings already described is confirmed.

The effect of hydrogen-ion concentration on the growth of water-hyacinth has been studied. There is an optimum range (6·0-8·0) of hydrogen-ion concentration in which water-hyacinth thrives. Any deviation from this range affects the growth adversely, large alteration resulting in the death of plants. Calcium salts probably antagonise the effect of hydrogen-ion concentration, and that is the reason why sometimes plants are not killed by changing the hydrogen-ion concentration.

Water-hyacinth is able to resist a considerable degree of drought and survives even when the water content of the soil falls as low as 5·7 per cent. of the saturation value. The resistance is due to :—

(*a*) change in the osmotic strength of the cells

(*b*) decrease in the ratio of the total leaf area to the total root length, and

(*c*) presence of mucilage in the leaf-base.

Effect of poisons, especially copper sulphate, were investigated. The lethal dose of copper sulphate was found to be 0·018 per cent.

Water-hyacinth plants growing in sub-lethal solution tolerate concentrations which are lethal to " uneducated " plants.

Application of copper-sulphate affects the roots and makes the absorption lag behind transpiration.

Calcium oxalate crystals escaping from disintegrating leaves antagonise the effect of copper sulphate. Laboratory experiments lend support to this view. This is perhaps the explanation of failures of spraying experiments on a field scale.

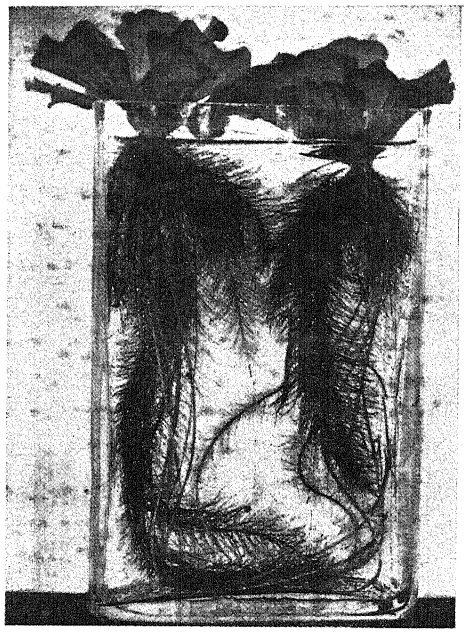


Fig. 1.—Control plants of *Salvinia cucullata* growing in ordinary tap-water.

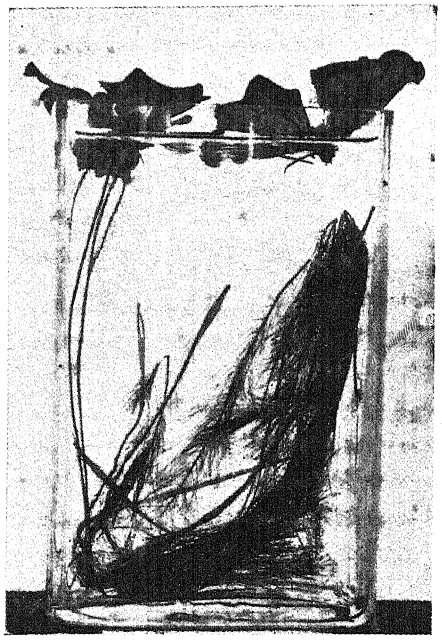
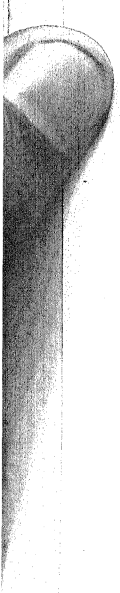


Fig. 4.—Experimental plants of *Salvinia cucullata* in copper sulphate solution, at the end of 48 hours.



Other weeds.

Pistia, which succeeds water-hyacinth in cleared tanks, was studied as regards its relative growth rate with water-hyacinth. Although the growth rate of *Pistia* was more or less similar to that of water-hyacinth, the former spreads on water surface more than the latter.

Germination of *Monochoria hastæfolia*, a member of Pontederiaceæ was studied, and was found very similar to that of water-hyacinth.

Stages in the germination of *Pistia* seed are recorded. Seeds germinate under water and float up. Spraying with kerosene makes them sink.

Effect of copper sulphate on *Pistia* was investigated. The lethal dose for this plant is much lower (0·006 per cent.) than that for water-hyacinth. On application of copper sulphate, the roots of *Pistia* fall off the plants and then the plants die.

Kerosene spray was tried on *Pistia* and *Salvinia* both of which are killed by it.

XI. Results of practical value.

1. From field observations and experiments in the garden it was seen that the water-hyacinth sets seeds only in the autumn, *i.e.*, in October and November. This fact is of great practical value. It has been the practice so far to search out seedlings during the early rains. On account of the fear that the pest left undisturbed may be a source of danger. The weed really becomes a perennial source of danger when it produces and discharges seeds into the mud. As the difficulty of distinguishing seedlings from other weeds is great, the weed may be left undisturbed till the middle of the rains and then cleared as the plants can then be easily located. In consequence of this finding, now the executive officers have been asked to see that the tanks are cleared before the end of September.

2. The seed lie dormant under water and mud for five years at least and do not germinate without access of oxygen. If the tanks do not dry up the seeds can not get the necessary oxygen for germination. Thus seeds may be prevented from germinating by flooding tanks. Seeds on tank sides lie buried in mud and when a shower of rain exposes them, they germinate freely, and this process goes on from year to year as more and more seeds get exposed. It has been suggested that quick germination may be effected by ploughing up tank sides and thus free them of the water-hyacinth more quickly. But it is very doubtful if this will be of any practical value.

3. Experiments were carried out on the effect of hydrogen-ion concentration. The conclusion drawn from the experiments is of considerable importance. There is an optimum hydrogen-ion concentration for growth of the plant. Any change to either side of the optimum checks the growth but does not kill the plants completely. If

the concentration changes towards the optimum, as it is likely to do in nature, the plants grow again. Occasional spraying, therefore, with suitable fluids is of no avail against the pest.

4. Experiments with copper sulphate proved most effective. A very low concentration (0·018 per cent.) proved injurious to the plants, but it was found that even in strong solutions all the plants were not killed. The reason was found to be the antagonism to copper sulphate of calcium salts liberated by the decaying water-hyacinth plants. This explains why sprays have not been always successful. The lesson from this is that vigilance and mechanical clearing is still necessary even after poisoning the plants.

5. Investigations into the drought resistance of water-hyacinth settled on land showed that the plant has the capacity of producing long roots with the progress of drying of the mud on which it settles. The only effective method to deal with plants settled on the tank sides is to plough them up and expose the roots and thus destroy them.

It is a common experience in these parts that *Pistia* succeeds in most tanks cleared of water-hyacinth. Although it was found that *Pistia* does not cut off light so completely as a thick growth of water-hyacinth does, still a thick growth of *Pistia* is obnoxious.

Experiments both in the garden and the field showed that *Pistia* can be effectively cleared by spraying with kerosene or better with a mixture of kerosene and copper sulphate. Sprayed with kerosene the plants spread, sink and rot, while copper sulphate (0·06 per cent.) makes the roots fall off, thus killing the plants.

6. The same methods can be used effectively against the water-fern, *Salvinia*, which forms matted growth in shallow stretches of water in these parts.

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Explanation of plates XXV-XXVII.

Plate XXV.

(All the microphotographs in this and subsequent plates have been taken with "Brownie" Camera with the box-camera-adapter devised by Mr. T. C. N. Singh to whom I am indebted.)

Fig. 1. A ripe capsule, with perianth covering intact.

Fig. 2. A ripe capsule dissected out: P=Perianth covering. C=Capsule; S=Ripe seeds exposed.

Fig. 3. Median longitudinal section of a ripe seed of water-hyacinth showing the fully developed embryo: R=Radicle; P=Plumule; V. S.=Vascular strand.

Figs. 4 and 5. Transverse sections through the plumule region.

Fig. 6. Longitudinal section of a seed through the micropylar region, showing the opening of the micropyle and a portion of the tip of the embryo: M=Micropyle; E=Embryo.

Fig. 7. Photomicrograph of the transverse section of a seed of water-hyacinth: E=Embryo; En=Endosperm; R=Ridges on the testa; L=Remnant of the dried flesh.

Plate XXVI.

Fig. 1. Water-hyacinth showing two bent spikes (B. S.), with fading flowers immersed in water, ready to discharge ripe seeds in the water, from ripe capsules.

Fig. 2. Water-hyacinth showing two erect spikes (E. S.), with flowers.

Fig. 3. Portions of two specimen-tubes containing water-hyacinth seeds germinated in water.

Fig. 4. A photomicrograph of a germinating seed, illuminated by the opaque illuminator, showing the radicle growing out from a side: R=Radicle; S=Seed; A=Remnant of the attachment of the seed to the inner wall of the capsule.

Plate XXVII.

(*Germination of seeds in the laboratory.*)

Fig. 1. Earliest stage—showing the tip of the radicle just protruding out through the micropyle.

Figs. 2 and 3. Showing the cotyledon growing out carrying with it the radicle and the plumule.

Fig. 4. The cotyledon has grown to its maximum limit. The hypocotyl is intercalated. The frill of transitory root-hairs is seen through which the radicle-tip is protruding out. The first leaf is seen coming out on a side: S=Seed; C=Cotyledon; H=Hypocotyl; R=Root-hairs; R. T.=Radicle tip; F. L.=First leaf.

Fig. 5. A later stage of fig. 4.

Fig. 6. A seedling in a later stage from moist soil without any intercalation of the hypocotyl.

Fig. 7. A photomicrograph showing the frill of root-hairs and the radicle growing out from the centre of the frill: R=Root-hairs; R. T.=Radicle-tip.

STUDY OF THE PECTIC CHANGES IN THE POTATO TUBERS AT DIFFERENT STAGES OF GROWTH AND IN STORAGE.

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(With three text figures)

INTRODUCTION.

In Western India, the problem of storing the potato has received great attention on account of the difficulty of keeping them in good condition during the hot weather. The potatoes need to be preserved at least for seed for the next season and there has been constant trouble experienced to prevent the rot setting in. The loss caused by rotting is enormous as during hot weather the whole stock of potatoes gets rotten in spite of proper aeration in storage rooms or in the field storage. The potato is cultivated in all parts of India. In the Bombay Presidency, it is cultivated in the Deccan and the Karnatak. It is cultivated in the Punjab, Rajputana, Central India, and on various hills like Mahabaleshwar, Simla, etc., and it is valued as an article of food by all classes of people. The rotting of potatoes in the hot weather necessitates its importation from Italy especially for the purpose of planting them in the next cold weather and therefore a study of the "black rot" of potatoes was made by various workers. In the Bombay Presidency Mann and his colleagues [1920] have made pioneer investigation on potato cultivation in Western India and they included the study of "heat rot" of the stored potatoes in their researches. They determined the conditions of storage which would result in the minimum amount of loss. They are (1) rigorous sorting and removal of the potatoes after fumigation showing the least signs of diseases, (2) storage of potatoes in upright standing single bags filled to the top in well aerated godowns, (3) the keeping of the temperature below 90°F. Mann and his colleagues [1920] found that the rotting of potatoes is not primarily caused by the attack of fungi or bacteria, but is caused by something which is intimately connected with the rise of temperature. They, therefore, determined the nature of the chemical

changes which accompanied the rotting of potatoes at higher temperatures. They determined the moisture, total soluble matter, total nitrogen, ammoniacal nitrogen, total insoluble and soluble proteid nitrogen, amido nitrogen, reducing and total sugars, gummy matter, acidity, alkalinity, catalases and oxidases in potatoes stored at 27—30° C. and at 36° C. They found that the total soluble matter, ammoniacal nitrogen, gummy substances, catalase and oxidase increase when the potatoes were exposed to 36° C.

Ajrekar [1924] however did not agree with the findings of Mann and his colleagues [1920] that the temperature was the cause of rottings which, according to his finding, was caused by bacterial diseases. Mann and his colleagues [1920] in their chemical studies of the "heat rot" or "black heart" of potato made practically a complete analysis of the contents of the potatoes stored at two different temperatures, but they unfortunately missed to determine the changes in the pectic constituents of the cell wall of the parenchymatous cells which might be responsible for the softening of the tissues of potato with the rise of temperature in the hot weather, which indirectly prepared the way for the progress of fungal and bacterial diseases. From the review of the pectic constituents of the cell walls by Branfoot [1929] it is clear that the pectic changes caused in the tissues by the activity of the enzymes present in the living cells, are responsible for the softening of the tissues and it is very likely that the same is true of the potato in sortage. If this be the case Mann and his colleagues [1920] on one side and Ajrekar [1924] on the other side are both right, the only difference being that they were looking at the two different sides of the shield.

Very little work has been done on the pectic constituents of the potato. Kellermann [1879] studied quantitatively the changes in the composition of growing potato and Czubata [1880] in a similar study of the frozen and rotten potatoes showed that half of the nitrogen constituents and the whole of the sugar disappeared in them. The composition of potatoes under different conditions has been studied by Konig [1880], Sacc [1884], Scovell and Menke [1887], Forfang [1904], in which various constituents of the tuber were determined and pectin was not estimated at all, or was estimated along with gummy substances. Hornby [1920] has estimated methyl pectate in the peel and flesh of the potato, Carre and Horne [1927] have included the potato in their microscopical study of the pectic substances in apples. They have found that the tissue of the potato is very compact with little intercellular space and the middle lamella is distinctly made of pectic substances. Goodwin and Martin [1930] have studied the chemical changes occurring during storage of potatoes from October to May. They did not observe any change in the composition of the dry matter until the sprouting of tubers when the protein content began to decrease and the reducing sugars began to increase.

They did not study the pectic constituents of the tubers. Applemann and Miller [1926] and Forts Chritte studied the loss in weight in potatoes during maturity and storage respectively.

The information, as the above review of the previous work shows, about the pectic constituents of potatoes, is both scattered and scanty, and no observations have been made about the pectic changes occurring in potatoes after harvesting. Potatoes are not morphologically comparable to fruits like apples or peaches. They may not be undergoing the stages of ripening in the sense that fruits like apples and peaches do. But it is possible that the same sequence of changes may occur in potatoes from the time of their formation to the physiological decay just as those found in other fruits, with this difference that the whole sequence of changes is not noticeable externally as it is in apples and other fruits. It is, however, noticed that the softening of potatoes occurs during the storage just as in the apples, and physiological death is as true of potatoes as it is true of other fruits. It is, therefore, considered possible that the same series of changes in the pectic constituents probably occurs in potatoes that are found in apples. The same pectic changes may also be similarly brought about by the fungus and bacterial diseases of potatoes just as they are found to be brought about by similar kind of diseases in apples.

In view of these considerations it was thought to be of interest as well as of importance to study the pectic changes occurring in potatoes from the time of their formation to their maturity and to extend the same observations to potatoes under storage till they become soft or till they show signs of softening on account of the fungus or bacterial diseases to which they are not immune. The study of these pectic changes going on in potatoes has not been made so far either in India or abroad and such a study may prove of practical value as the problem of storing potatoes is so very acute in India during the hot weather. If it can be shown that the softening of potatoes is caused by the greater enzymatic activity on pectic constituents of the cell walls or middle lamella with the rise of temperature, and that the vigorous growth of fungus and bacteria is nearly the result of these changes, it is possible to arrive at some practical conclusions about selecting potatoes for storage so as to avoid rotting during the hot weather.

The present investigation is confined to (1) the methods of extraction and estimation of different pectic constituents from potatoes, (2) the study of pectic changes occurring in potatoes from the time of their formation till maturity and in storage till the physiological death or death caused by fungus diseases, and (3) the study of the effect of temperature on the pectic constituents of potato tubers.

Investigation.

The potatoes used in this investigation were obtained from the following sources :

(1) The Italian oval variety that is imported into Bombay in October.
(2) Potatoes (from Italian round seed) in various stages of formation were obtained from the Agricultural College, Poona.
(3) Potatoes grown from the Italian oval seed at Deesa (Palanpur State, Rajputana).
(4) Potatoes grown from Simla Seed at Deesa were obtained in various stages of their formation.
(5) Potatoes grown from the Italian round seed at Telegaon, Poona District.

Methods.

The important modifications that the pectic compounds undergo necessitated the development of the methods for the separate quantitative extraction of the various forms of the pectic material. Rosenberg attempted the extraction of protopectin and pectic material in the middle lamella and the decomposition products of pectic substances during ripening separately. Fellenberg [1918] followed Rosenberg ; but Carre and Haynes [1922] found that water can substitute the sugar solution with equal accuracy. Carre and Haynes [1922] developed quantitative methods for the separate extraction of the various pectic compounds, making use of the different chemical properties. Tutin [1921] extracted protopection with boiling water after immersing certain leaves in boiling alcohol. Nanji and Norman [1928] have tried to develop, on the lines of Carre [1922], the quantitative methods for the different pectic compounds. During the present investigation, the method of Nanji and Norman [1928] for the extraction of the soluble pectin is found inaccurate as water at 85° C. extracted much of the protopectin from the dried material.

The extraction of protopectin by prolonged heating with 0·5 per cent. oxalic acid at 85° C. was discarded by Codling and Woodman [1930] and they definitely advocated the extraction of the pectic substance with acids by instalment and not by prolonged heating. Emmett [1929] has improved Carre's method [1922] for the middle lamella pectic material pointing out the inadvisibility of the use of *N*/75 sodium hydroxide at boiling temperature while Branfoot [1929] has introduced 0·5 per cent. ammonium oxalate for *N*/75 sodium hydroxide as suggested by Nanji and Norman [1928] for total pectic material.

Soluble pectin.—Potato tuber was pulped with a food-chopper and the beaker with the pulp was cooled in freezing mixture for one hour, and then allowed to attain the room temperature. The pulp was extracted with cold water by crushing in a pestle and mortar the mixture being every time transferred to a piece of cloth spread on a beaker and then strained. These extractions were continued till the last extract, after filtering through the filter paper, gave no precipitate with acidified alcohol. Usually ten to fifteen such extractions were sufficient, as much of the soluble pectin comes in the juice of the cell-sap.

Link and Tottingham [1923] have studied the effect of various methods of desiccation of potato tubers and they give the best conditions as heating at 80° C., and 35 mm. pressure, in an unventilated oven for 12 hours, when the material is apparently not discoloured and its odour gives no evidence of caramelisation. The sprouting tubers only were autoclaved for fifteen minutes at 116° C. and 16 lbs. pressure to inhibit enzyme action.

The potato tuber was sliced transversely into pieces six to seven millimetres thick. For sprouting tubers autoclaving was resorted to in a Kenvers' cooker in a beaker covered with a glass dish under the above-mentioned conditions. The autoclaved or unautoclaved material, as the case may be, was placed in a pressure-filter flask and dried at 85° C. with 45 mm. pressure. The effect of drying at 98°C. and normal pressure as followed by Nanji and Norman [1928] was tried, and it was observed that the pectic content was affected. The dried material was powdered to 60 mesh fineness by first grinding in a coffee grinder, then sifting in graded sieves and reducing the residue to the same mesh by the use of pestle and mortar.

Extraction of protopectin.—The finely powdered material (1·5 grm.) was placed in a round bottomed flask and about 40 c.c. of boiling water were poured on it and kept for five minutes to remove the soluble pectin as followed by Emmett [1929] and filtered through a Buchner. The material was scraped from the filter paper with a nickel spatula and washed with *N*/75 hydrochloric acid in a round bottomed flask. The total quantity of acid used for one extraction was fifty c. c. The round bottomed flask with its contents was immersed in a boiling water bath for three hours with a reflux condenser. The extraction was repeated twice and the residue was washed with hot water till the last washing gave no precipitate with acidified alcohol.

Extraction of total pectin.—The finely powdered material (1·5 grm.) was placed in a round bottomed flask with 50 c. c. of 0·5 per cent. ammonium oxalate and refluxed in a water bath at 85° C. for 36 hours. The optimum period for the extraction of total pectin in the present investigation was 36 hours as the residue after this period of extraction gave no precipitate with further ammonium oxalate extract and acidified alcohol, but the residue gave pale red colouration with ruthenium red and did not completely dissolve in Schweitzer's reagent. After the extraction period the mixture was transferred to a beaker and washed with boiling water till the last filtrate gave no precipitate with acidified alcohol.

For the quantitative estimation of the pectic compounds, the method of converting them into insoluble calcium pectate is generally adopted. The results obtained by this method represent the relative amounts of pectic materials present in terms of their common basal molecule (pectic acid) invariable in composition. The calcium pectate method of estimation does not give an absolute measure of the pectic compounds owing to their variable composition and heterogeneous character. The precipitation of pectin becomes increasingly difficult with the increase in dilution. As pectin dilution increases, the precipitate becomes increasingly sticky in character due to the increase of pectin in the soluble phase, thus adding to the difficulties of filtration and precipitation. Thus the pectic coagulum is not necessarily homogeneous. Wichmann [1924] advocated the alcohol process, while Hardy [1924] gave results of calcium pectate and alcohol methods, showing every time the alcohol precipitate to be higher due to impurities. Estimations based on the methyl alcohol yield suggested by Fellenberg [1917] and employed by Hornby [1920] or those based on the acetone liberation of Dutt are inaccurate, as pectin in all natural sources is presupposed to have the same methoxy content and is the source of all methoxy groups in vegetable tissues. This is not true as other substances in the plant tissue may have methoxy groups in their composition.

Estimation by calcium pectate method.—Only for the soluble pectin, the extract was heated and filtered before precipitation. An aliquot portion of the extract from any of the extractions (usually 1/4th of the total volume of 200-250 c. c. containing 0·008-0·015 grm. calcium pectate) was precipitated with three volumes of 95 per cent. alcohol as recommended by Buston and Kirkpatrick [1931], acidified with hydrochloric acid (usually 15-20 drops) required to make the resulting mixture *N*/10. The colloidal solution of pectin in alcohol is precipitated by an electrolyte like hydrochloric acid or sodium chloride. The acidified alcohol was used as it precipitated mostly the pectic substances without precipitating other colloidal substances on account of higher hydrogen-ion concentration in acidified alcohol than in pure alcohol. It was also observed that the addition of acid to alcohol beyond a particular limit resulted in decreasing the precipitate. This observation did not support the conclusions of Nanji and Norman [1928] that the concentration of acid in alcohol did not affect the yield. The extract was

not concentrated before precipitating with alcohol as suggested by Nanji and Norman [1928] as it resulted in a decrease in the yield of precipitate as calcium pectate. Next day the precipitate was filtered in a fluted filter paper, washed once with acidified alcohol (75 per cent.) and dissolved off the paper with as little boiling water as possible and filtered. It was then hydrolysed with 40 c. c. $N/10$ sodium hydroxide by keeping the mixture overnight. Next day 25 c. c. of normal acetic acid were added and after five minutes 25 c. c. $M/1$ calcium chloride were added when calcium pectate came down in the form of flocculent precipitate. The mixture after one hour was boiled for a few minutes (at least for five minutes), decanted twice in a weighed Gooch's crucible dried at 100° C., then the precipitate was transferred and washed with boiling water till the filtrate gave no precipitate with silver nitrate. The precipitate was dried at 100°C. for twelve hours to constant weight. The addition of ammonia to dissolve the alcohol precipitate which might contain pectic acid as suggested by Nanji and Norman [1928] was not found necessary in the present investigation.

Quantity of the middle lamella pectin in the tubers is obtained by deducting the values for protopectin and soluble pectin obtained from that of the total pectin.

The results in the present investigation are expressed as yield of calcium pectate per 100 grms. of dry material, *i.e.*, the calcium pectate number of Nanji and Norman [1928]. All the results recorded are the average of two determinations.

Results and their discussion.

According to the methods described above the different pectic constituents were determined in potatoes grown from Simla seed at Deesa at different stages of formation. It is difficult to assign the age or stage of formation of a potato from external examination or according to the date of collection from the field. The only criterion left to judge the age of a growing tuber is its size provided it is growing normally and its growth is not arrested due to various physiological factors. The potatoes of different diameters were collected personally from the field from plants of normal growth and a large number of each size was collected in order to avoid errors arising out of uncontrollable conditions. Nine different stages of growth of potatoes are arbitrarily chosen according to their diameter and they include tubers of all stages from the beginning of their formation till maturity (*i.e.*, suitable for harvesting).

The determinations of the soluble pectin in these tubers are made soon after their collection in order to find out the changes in the soluble pectin as the tubers grow. If the determinations are delayed, secondary changes may occur in the pectic constituents. Table I gives the free soluble pectin, protopectin, middle lamella pectin and the total pectin, expressed as calcium pectate number. The fresh weight of the potato taken for each analysis is about 20 grms. The results clearly indicate a rise of soluble pectin in tubers as they grow. The quantity of soluble pectin is nearly doubled in the ninth stage of growth of the tubers as compared with that of the first stage. The graph showing the changes in the soluble pectin, as the tubers mature, is given in Fig. 1. The increase in the free soluble pectin is very gradual as the graph clearly indicates.

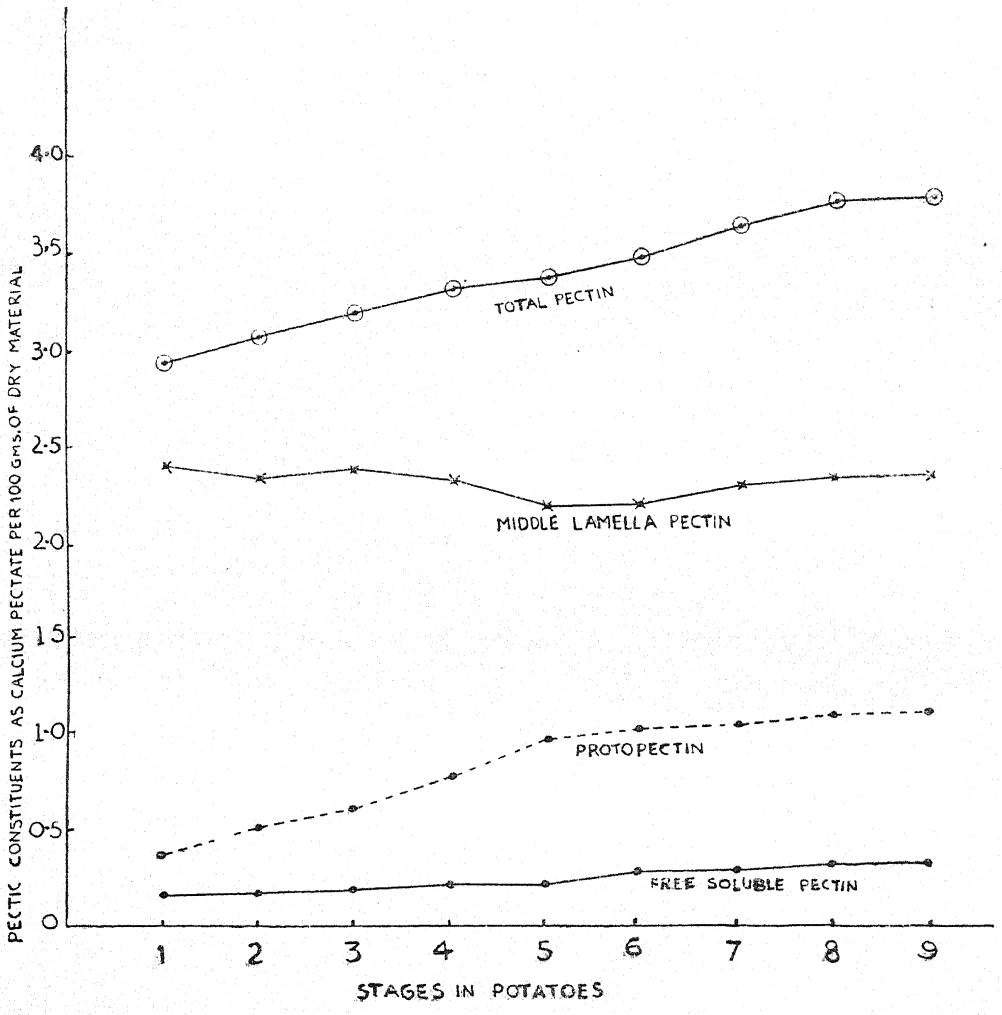


Fig. 1. Free soluble pectin, protopectin, middle lamella pectin and total pectin in potato tubers (Simla variety, Deesa crop) of different stages of growth.

The protopectin also increases as the growth of the tuber proceeds. This indicates that the growth is active and the increased amount of pectic formation occurs as the potato matures. The quantity of protopectin is always much higher than the quantity of soluble pectin at each stage of growth. The graph showing the rise in protopectin is given in Fig. 1. In the first five stages the increase of protopectin is very rapid as the steep nature of the curve shows and in the last stages the curve becomes parallel to the abscissa. The middle lamella pectin

remains constant in all the nine stages of growth of the potato tubers, except for a slight decline in the fifth and sixth stages of growth. This is explicable on the supposition that the pectic materials in the tubers analysed for these two stages have already begun decomposing. This view is supported by the fact that there is a great rise in the soluble pectin at those stages (Table I). The graph showing the changes in the middle lamella pectin is given in Fig. 1.

TABLE I.

Free soluble pectin, protopectin, middle lamella pectin and total pectin as calcium pectate number in potato tubers (Simla seed; Deesa crop) in different stages of growth.

Stage of potato	Average diameter of potatoes in cm.	Free soluble pectin	Protopectin	Middle lamella pectin	Total pectin
		(Average of two determinations)			
I	1·0—1·4	0·1577	0·3520	2·400	2·910
II	1·6—2·0	0·1601	0·5100	2·343	3·024
III	2·2—2·6	0·1863	0·6036	2·383	3·173
IV	2·8—3·0	0·2190	0·7610	2·317	3·297
V	3·1—3·3	0·2213	0·9385	2·189	3·349
VI	3·5—4·0	0·2705	1·0090	2·190	3·460
VII	4·2—4·6	0·2759	1·0440	2·290	3·610
VIII	4·8—5·2	0·3028	1·0970	2·330	3·720
IX	5·3 onwards.	0·3034	1·1070	2·340	3·750

There is distinct rise in the total pectic substances as the potato tubers grow. The pectic constituents on the whole increase from the first stage to the ninth stage of growth though the percentage of the pectic constituents of the middle lamella remains constant. The graph showing the changes in the total pectic material is given in Fig. 1. Most of the pectic material is found in the middle lamella of the cell walls. Eighty-two per cent. of the total pectic material is in the middle lamella in the first stage of growth and the amount decreases to 62 per cent. in the ninth stage. Similarly the percentage of free soluble pectin and protopectin in the total pectic material increases as the growth proceeds.

Similar determinations of the free soluble pectin, protopectin, middle lamella pectin and total pectic materials in the tuber of different stages of growth grown at Poona from Italian seeds are made to study the sequence of changes occurring in the tubers during growth and to see if there are any differences noticed as compared to the tubers grown from the Simla seed at Deesa. The tubers of different stages of growth as differentiated by the differences in their size were kindly sent by the Principal, Agricultural College, Poona.

TABLE II.

Free soluble pectin, protopectin, middle lamella pectin and total pectin as calcium pectate number in potato tubers grown at Poona (Italian Round Seed) at different stages of growth.

Stage of potato	Average diameter of potatoes in cm.	Free soluble pectin	Protopectin	Middle lamella pectin	Total pectin
		(Average of two determinations)			
I	0·6—1·25	*nil*	0·3002	2·509	2·809
II	1·26—1·87	*nil*	0·5983	2·346	2·944
III	1·87—2·5	*nil*	0·6495	2·469	3·119
IV	2·6—3·12	0·1534	0·5675	2.517	3·2365
V	3·13—4·12	0·1537	0·6058	2·535	3·295
VI	4·2—5	0·1994	0·6405	2·505	3·345
VII	5—6·2	0·2079	0·7485	2·452	3·407
VIII	6·3 onwards.	0·2247	0·9952	2·325	3·545

The free soluble pectin, protopectin, middle lamella and total pectin at different stages of growth of the tubers are given in Table II. The study of the results shows that the similar changes in the different pectic constituents occur in these tubers as they grow as was found in the Deesa crop. Free soluble pectin is absent in the first three stages of growth. There is a slight decline in the middle lamella pectin. The total pectins increase as the age advances. The middle lamella pectin forms the largest bulk of the total pectin in growing potatoes. Free soluble pectin and protopectin increase as the tubers grow in size.

Having studied the sequence of the pectic changes occurring in the tubers during their growth it was undertaken to study the sequence of the pectic changes occurring in the same tubers which were stored up in a well-ventilated wooden box at the room temperature during the hot weather. The stored potatoes were analysed every fortnight from June till September. It must be pointed out here that though the sequence of changes occurring in potatoes under storage may be the same for all, yet it is learnt after a good deal of experience that all the tubers of a variety grown at the same place do not pass through the same series of pectic changes at the same time. In some the changes occur with greater rapidity than in others. These differences are not noticeable on the external examination of the tuber and consequently a great deal of error is introduced in the estimations of pectic changes as potatoes in different stages of pectic changes are unavoidably selected on each date. For these reasons sufficient care was taken in selecting potatoes for each analysis so as to minimise the error as much as possible. The

potatoes (Simla seed grown at Deesa) were stored up in Bombay during the hot weather and the pectic changes were studied in them.

Table III gives the results of pectic analysis of potatoes from June to September.

TABLE III.

Free soluble pectin, protopectin, middle lamella pectin, and total pectin as calcium pectate number in potatoes (Simla seed) grown at Deesa.

Date of determination	Free soluble pectin	Protopectin	Middle lamella pectin	Total pectin
	(Average of two determinations)			
1932	*Stored in Bombay.*			
20th June	0·3654	1·333	1·443	3·141
5th July	0·4452	1·595	0·827	2·867
20th July	0·4830	1·423	0·803	2·710
4th August	0·4698	1·330	0·880	2·680
19th August	0·6633	0·870	0·900	2·433
3rd September	0·7224	0·797	0·748	2·267
	Stored at Deesa.			
16th July	0·3987	1·641	0·964	3·004
28th July	0·4318	1·315	1·143	2·890
11th August	0·4722	1·175	1·180	2·826
27th August	0·5383	0·862	1·163	2·557
12th September	0·6786	0·575	0·920	2·174
27th September	0·7151	0·444	0·826	1·985

The free soluble pectin continues to increase as the age advances. This increase in the soluble pectin is maintained from the very beginning of the tuber-formation till the potatoes approach the stage of senescence or death due to the attack of fungi or bacteria. The graphs showing the changes in the free soluble pectin and protopectin are given in Fig. 2. The insoluble protopectin shows a decrease. The fall in the protopectin may be causing the rise in the free soluble pectin. The middle lamella pectin and total pectin also decrease with time (Fig. 2), (Table III).

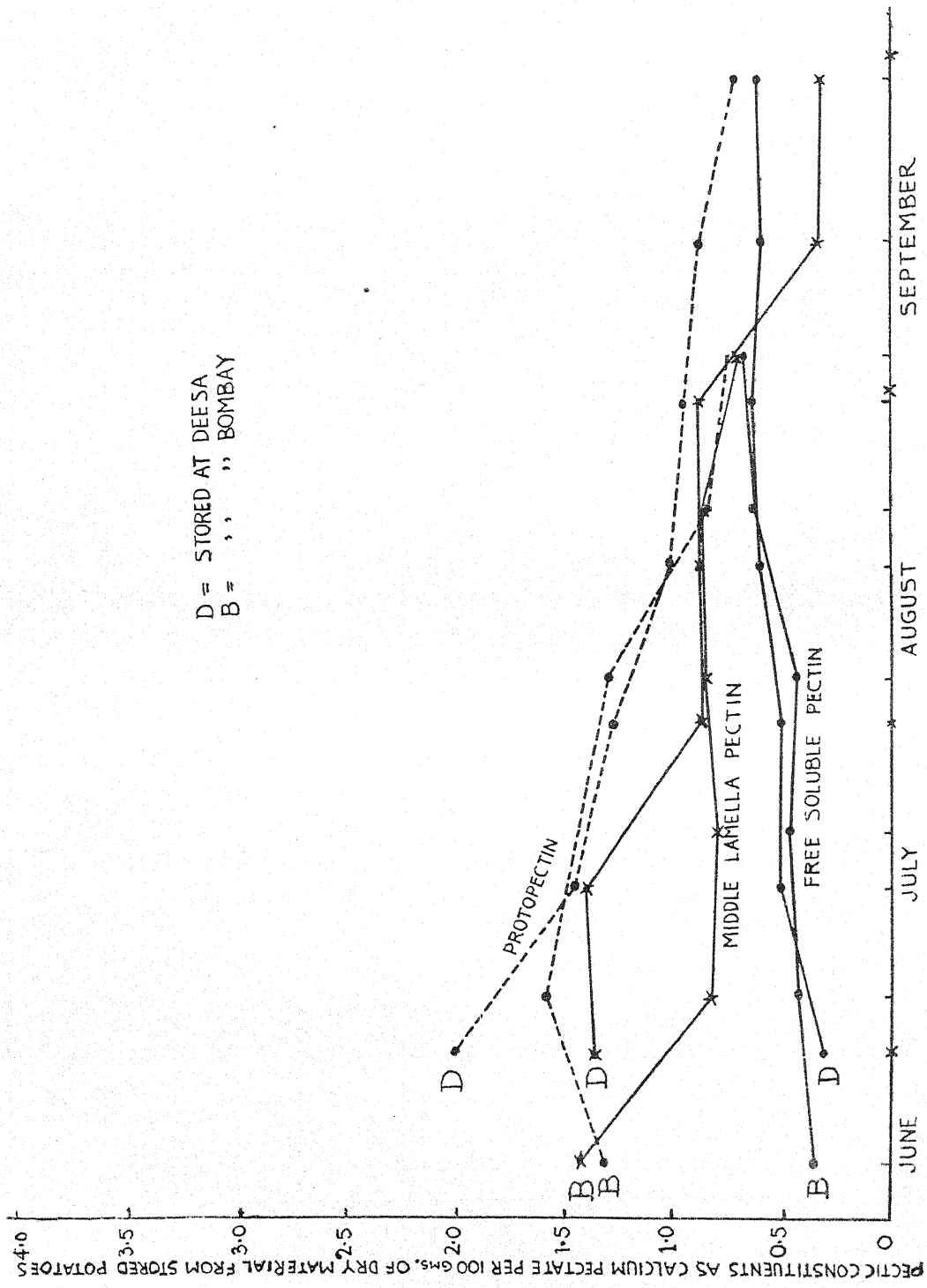


Fig. 2. Changes in the free soluble pectin, protopectin and middle lamella pectin in the stored potato tubers.
(Simla seed stored in Bombay and Deesa from March to June.)

A second lot of the same kind of tubers grown from the Simla seed at Deesa was sent for directly from the potato godowns at Deesa in June 1932 to study the changes in the pectic constituents of the potatoes kept there during hot weather. The temperature at Deesa goes up to 120° F., and it would be interesting to study the changes in the pectic constituents of the tubers stored there during the hot weather. Table III gives the results of the analysis of the pectic substances from July till September 1932. The results show that the free soluble pectin increases from 0·3987 to 0·7151 and there is a marked fall in protopectin to nearly one third of the original value. The middle lamella pectin and the total pectin decrease on the whole. (The first result has a low value of middle lamella pectin on 16th July 1932; but this may be due to the causes mentioned above.)

The changes in the pectic materials of tubers grown from Italian oblong seed at Deesa were also investigated. The tubers were obtained from Deesa where they were harvested in March 1932 and stored up in local godowns. It would be interesting to study the changes going on in tubers raised from the imported seeds and compare these changes with those occurring in potatoes raised from the seeds in India. The following table gives the different pectic constituents of these tubers as before. The results obtained with the Italian variety of potato agree with those obtained with the Simla variety of potato. The free soluble pectin increases from 0·3262 to 0·7378 and protopectin decreases from 2·088 to 0·488. The amount of protopectin is greater in the Italian variety than the quantity of protopectin in the Simla variety. The middle lamella pectin decreases as before with time. It decreases from 1·362 to 0·480. The fall is very great as compared with the fall in the previous series. The total pectic material decreases from 3·776 to 1·707. The fall in the total pectic material is also very marked.

TABLE IV.

Free soluble pectin, protopectin, middle lamella pectin and total pectin as calcium pectate number in potato tubers (Italian variety).

Date of determination	Free soluble pectin	Protopectin	Middle lamella pectin	Total pectin
	(Average of two determinations)			
1932	*Grown and stored at Deesa.*			
30th June	0·3262	2·088	1·362	3·776
15th July	0·5091	1·463	1·401	3·374
30th July	0·5203	1·302	0·880	2·653

TABLE IV—*contd.*

Date of determination	Free soluble pectin	Protopectin	Middle lamella pectin	Total pectin
	(Average of two determinations)			
1932	*Grown and stored at Deesa*—contd.			
14th August	0·6515	1·042	0·906	2·600
29th August	0·6897	0·986	0·925	2·600
13th September	0·6563	0·930	0·394	1·974
28th September	0·6782	0·776	0·387	1·843
14th September*	0·7292	0·497	0·427	1·654
29th September*	0·7378	0·488	0·480	1·707
	Grown at Talegaon and stored in Bombay.			
25th June	0·3571	1·345	0·799	2·507
10th July	0·3940	1·256	0·671	2·321
25th July	0·4928	1·201	0·320	2·013
9th August	0·5600	1·118	0·5085	2·182
24th August	0·6700	0·917	0·3995	1·982

* The determinations marked thus * in the above table are taken for rotten potatoes.

The determinations of the different pectic constituents in potatoes of the Italian variety grown at Talegaon were also made. The potatoes were obtained locally from a potato godown and the determinations from June to September were made. The results of the free soluble pectin, protopectin, middle lamella pectin and total pectin are given in Table IV. These results also confirm the above results in the case of the Italian variety grown and stored at Deesa.

The main conclusions that can be drawn from the results are that the changes in the pectic substances occur from the beginning of the formation of the tubers up to their senescence and decay; and that they occur in regular sequence as described above. The protopectin undergoes changes while the free soluble pectin increases in amount. The total pectic constituents also show a decrease indicating that pectin decomposes into pectic acids which ultimately decompose into sugars. The decreasing amount ot protopectin which is supposed to be combined with cellulose in

cell walls indicate that in some way or other the degradation of protopectin occurs as the age advances and consequently brings about a loosening of the cell walls. This loosening of the cells is indicated by the softening of potatoes. These changes in the pectic constituents, it appears, are affected like all other chemical changes by temperature and they are accelerated during the hot weather when the temperature is higher than in other months. The loosening of the cell wall caused by the changes in its pectic composition makes the tuber more susceptible to the attack of bacterial and fungal organisms which are always present under the conditions of storage and consequently they hasten the progress of decay of the tubers.

In order to prove that temperature has an accelerating effect on the pectic changes of potatoes, a series of experiments were performed with freshly harvested potatoes of the Italian variety. In these experiments potatoes were subjected to two different temperatures for different periods of time every day for seven days, and the pectic constituents analysed at regulated intervals. The tubers were heated for six hours during the day from 9 a.m. to 3 p.m. for seven days successively at 40° C. The period of six hours only during each day was chosen as the potatoes in storage are also exposed to higher temperatures for about the same period during the day. Tubers were exposed in an incubator (Hearson's gas incubator) which was regulated at 40° C. (104° F.) at 9 a.m. on the 18th November 1932 and taken out at 3 p.m. on the same day and kept at room temperature which fluctuated between 25° and 29·5° C. The same process was repeated every day up to 24th November 1932. The tubers were analysed every sixth day beginning with the 25th November. Side by side unheated tubers stored at room temperature were also analysed every sixth day, following the one for the analysis of the heated ones (tubers). The results of analysis are given in Table V. The free soluble pectins in the unheated and heated potatoes at different dates are given in Table V, and Fig. 3 indicates the changes in them. In the unheated tubers the free soluble pectin shows a rise from 0·4115 to 0·4633, while in the heated tubers from 0·4124 to 0·7245 during the same period. Similarly the protopectin decreases in the unheated potatoes from 1·336 to 1·167 and the heated potatoes from 1·361 to 0·549. The graph showing the changes in the protopectin is given in Fig. 3. The change in the middle lamella pectin is from 1·294 to 0·945 in the unheated tubers and from 1·194 to 0·286 in the heated ones. The middle lamella pectin shows an abrupt fall during the last period from 7th December to 13th December 1932, and the fall is very marked as shown in Fig. 3. The total pectic materials decrease from 3·041 to 2·524 in the unheated tubers and from 2·973 to 1·559 in the heated ones, and the graph showing the changes in the total pectic materials is given in Fig. 3. The above results indicate that a high temperature hastens the decomposition of protopectin and middle lamella pectin into free soluble pectin and very probably pectic acid.

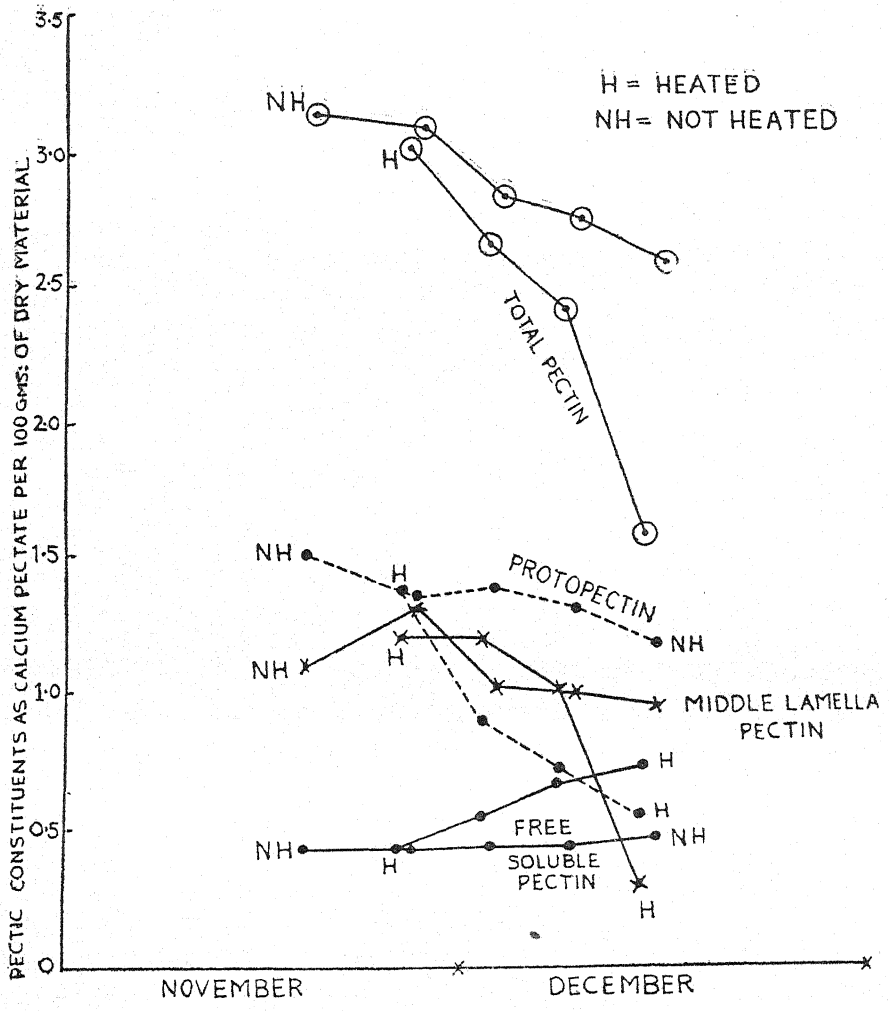


Fig. 3. Changes in the free soluble pectin, protopectin, middle lamella pectin and total pectin in potato tubers, heated at 40°C. and in unheated tubers.

The potato tubers in the next experiments were exposed to a temperature of 36° C. (97° F.) for six hours during the day from 6 a.m. to 12 noon for successive seven days from 18th December to 25th December 1932. The fluctuation of room temperature was 25·5 to 30° C. The analysis of heated and unheated potatoes was done as in the former set of experiments every sixth day. The results are given in Table V ; and similar though smaller differences in the pectic constituents of the unheated and heated tubers as shown in Fig. 3, are found.

TABLE V.

Free soluble pectin, protopectin, middle lamella pectin and total pectin as calcium pectate number in tubers (Italian seed) imported in Bombay in November 1932.

Date of determination	Free sol. pectin	Proto-pectin	Middle lamella pectin	Total pectin	Free sol. pectin	Proto-pectin	Middle lamella pectin	Total pectin
	Average of two determinations.							
	Heated at 40°C.				Unheated*			
18th November 1932 .	...	...	...	...	0·4096	1·497	1·187	3·093
25th ,, ,, .	0·4124	1·361	1·194	2·973	0·4115	1·336	1·294	3·041
1st December 1932 .	0·5383	0·889	1·199	2·627	0·4238	1·362	1·015	2·801
7th ,, ,, .	0·6586	0·715	1·011	2·384	0·4265	1·294	0·999	2·719
13th ,, ,, .	0·7245	0·549	0·286	1·559	0·4633	1·164	0·945	2·574
	Heated at 36°C.				Unheated			
26th December 1932 .	0·5311	1·142	0·927	2·600	0·4661	1·054	1·133	2·663
1st January 1933 .	0·6234	0·897	0·906	2·227	0·5378	0·769	1·219	2·507
7th ,, ,, .	0·6641	0·773	0·733	2·169	0·5396	0·634	1·209	2·467
13th ,, ,, .	0·7360	0·491	0·662	1·893	0·6770	0·493	0·683	1·853
19th ,, ,, .	0·5884	0·305	0·360	1·254	0·5514	0·369	0·587	1·507

* The analyses of the unheated tubers were done on days following those for the heated tubers.

CONCLUSIONS.

The results of the analysis of the pectic constituents of the potato tubers from the very commencement of the tuber formation up to the senescence stage indicate that there is a regular sequence of changes occurring in them and it can be concluded that these changes are probably caused by the activity of the different enzymes.

The free soluble pectin during the formation stages of the tubers is very little in amount up to the mature state when it begins to increase. The rise in the free soluble pectin is very gradual and its presence in the immature tubers could only be explained in two ways : (1) either the free soluble pectin is secreted by the protoplasm and deposited in the cell walls and therefore it may be an up-grade free soluble pectin ; or (2) the decomposition process of the protopectin sets in from the

very beginning and continues upto the end of the senescence stage. In the latter explanation, the free soluble pectin present during the formative stages of the tubers is a down-grade pectin. The former view is more reasonable as the decomposition process is not likely to set in so early which would interfere with the normal growth of the tubers and secondly there is a continuous rise in protopectin during these early stages indicating their formation from the basic material which may be soluble pectin or some other simpler substances from which it is produced.

But it is certain that the decomposition process sets in early and it may set in before the tubers reach the normal size, or before they are harvested. It is impossible to say from external examination whether the down-grade process has started or not. This is the crux of the whole problem which, if solved, would prove of economic value as then it would be possible to store those tubers in which the decomposition has not already set in.

The protopectin is continuously on the rise as the tuber grows. It increases from 0·352 to 1·107 (Table I). During the last two stages the increase is very little as compared to the increase in the earlier stages and it may be due to the ordinary sequence of growth which in all plant-organisms begins slowly, increases rapidly and then begins to fall. In Table II the rise in the protopectin is very high in the last stage, but that may be due to the tubers analysed in the seventh stage being possibly in the younger stage of formation than the tuber analysed in the last stage. It is likely that the stages of growth of the two sets of tubers given in Tables I and II do not correspond. This is supported by the results of the free soluble pectin also in the two cases.

The middle lamella pectin does not show such serial rise in the formative phases of the tubers. It is preponderatly high from the very beginning and remains more or less constant at the end of the formative stage. The total quantity increases but the percentage increase is *nil*. The amount of middle lamella pectin added is commensurate with the increase in dry weight of the tuber. The middle lamella pectin is first laid down as the cells divide and there is no impregnation of pectic material as is very probably the case with protopectin. The total pectic material also increases as the tuber grows indicating the formation of pectic substances and this is due to the activity of the protoplasm of the cells.

Potato tubers provide excellent material to study the formation of pectic substances in plants as the results clearly show that they are actually produced in them and consequently they will supply us with the information about their actual mode of formation in plant-organs.

It is also of interest to find that although potato is a ' tuber ' the pectic changes are more or less similar to those taking place in stored fruits.

All these results discussed so far show that the maturity of a tuber coincides with the cessation of the up-grade pectic activity and the former can be determined for the potato tubers of different varieties by determining the total pectic materials provided there is no interaction of other factors.

Study of the results of the pectic analysis of the tubers stored in Bombay or at Deesa again reveal a sequence of uniform changes which are all of a down grade character as the protopectin which was on the increase in the formative stages is on a decline and the middle lamella pectin which had remained constant in the formative stages also shows disintegration of its substance. The total pectic materials are similarly on their down-grade path. These down-grade changes become rapid with the advance of age. These changes in the pectic constituents become more and more pronounced as the tubers decay and as their tissues soften. The cementing influence exerted by the presence of these pectic constituents on the cells is diminished gradually and the loosening of the cell walls of the tuber which were in close contact with one another occurs. So the pectic constituents play the roll of organic binding materials and they give rigidity and strength to the tissues ; and as they deteriorate, the tissue collapses and falls a prey to the attack of bacterial and fungal diseases whose spores are always present on the skin and in storage places. These organisms get a footing as it were when the tissues soften and hasten their decomposition by their destructive activities. Even if the tubers are not attacked the tubers will undergo their normal sequence of changes.

The rise of free soluble pectin in the tubers in storage is a down-grade substance evidently arising as a result of decomposition of protopectin and middle lamella pectin. But the fall in middle lamella pectin and protopectin is not balanced by the rise in the free soluble pectin. This is also evident from the fact that the total pectic materials decrease in amounts. It is likely that the free soluble pectins undergo further decomposition to simple substances like pectic acids. The presence of acidity in the tubers under storage is verified by the authors though the quantity is very small. The difficulty is that there is no direct method of determining the pectic acid as such. It is likely that the pectic acid also decomposes into arabinose and galactose which are in turn converted into carbon dioxide.

It is a common observation that all the tubers in storage are in the different states of senescence or decay although they may be harvested or stored at the same time. Some will show advanced stages of decay while others will be at earlier stages of decay ; yet others may not show decay at all except for a shrinkage of skin and little softening of the general surface. These indicate that the down grade pectic changes do not proceed at the same speed in all these tubers, some being in the advanced stages of pectic decomposition while some in the early stages. These assumptions are supported by the experimental data obtained. This raises a point

of importance. Is it possible to select such tubers for storage in which the pectic decomposition will set in later so as to tide over the period of storage without impairing their sprouting capacity ? This is only possible if we can find some general principle which should underlie the selection of tubers for storage. The general principle is, what is already mentioned above, the selection of tubers in which the down-grade pectic changes have not set in. The difficulty is how to apply the principle in practice. The following is a tentative suggestion in that direction. The pectic analysis of the fully formed tubers attached to the mother plants should be made periodically during the period before harvesting and the stage and time when the up-grade changes cease can thus be determined. If harvesting for storage purposes is done at that time, it is very likely that they will stand the period of storage better than the tubers harvested later when the down-grade changes have already commenced.

It must have been noticed in the experiments done on the influence of temperature on the pectic changes in the tubers that the down-grade pectic changes are accelerated by the rise of temperature and the quantity of protopectin, middle lamella pectin and total pectic materials have decreased more rapidly in the heated tubers than in the normal unheated ones kept at the room temperature. The greater effect of temperature on heated tubers at 40° C. is also visible as compared with the heated tubers at 36°·C. Similarly in nature, the temperature during hot weather is high and the high temperature accelerates the rate of downward pectic changes. These results indicate that the changes in the pectic substances that occur in the tubers are governed by chemical laws and like all chemical reactions they are accelerated by temperature.

It is also likely that the enzymes play their part in bringing about these decompositions of pectic materials and these enzymes are found to be present in decaying fruits. It is very likely that the enzyme action is accelerated by temperature and therefore the rates of the chemical reactions are also accelerated. Now in potatoes which are stored when the downward pectic changes are not set in, they will undergo lesser down-grade changes with the rise of temperature than those in which the down-grade changes have already commenced before harvesting. Because in the former case the decomposing enzymes are activated at later stages than in the latter, where the enzymes have already begun to act, the former tubers will undergo the down-grade pectic changes at the same rate as the latter ones and consequently they will decay later. It would be interesting to study the changes in the pectic constituents of tubers harvested on different days and also the changes in the harvested tubers which are slightly immature as well as in the fully matured ones.

In the work done on the storage of potatoes this important point has not been noticed before, as such systematic study of the pectic changes in tubers at different stages of growth and storage have not been attempted. It appears that if, by some means or other, it is made possible to select tubers which are free of these down-grade pectic changes, the problem of potato storage will be on its way to solution. It would even not be necessary to have recourse to the costly method of cold storage if this effort proves successful as it is common experience that some tubers do survive even under ordinary conditions of storage.

Summary.

The study of the pectic changes in potatoes at different stages of growth, maturity and of senescence during storage was undertaken as it appeared from the survey of the literature on the pectic constituents of fruits and other plant-organs that the softening of the tissue and its ultimate decay was due to the pectic changes occurring in them. The problem of potato storage is of economic importance in Western India and inspite of the efforts of the various workers there is no unanimity of opinion about the causes of decay of potatoes when stored during the summer months.

The methods of Carre and Nanji [1928] modified slightly for the needs of this investigation for extracting and estimating different pectic constituents were employed. The free soluble pectin, protopectin, middle lamella pectin and total pectic materials were determined and expressed as calcium pectate number for the tubers at different stages of growth and at short intervals after storage till the tubers completely rotted.

The free soluble pectin, protopectin, middle lamella pectin and total pectins begin to rise as growth proceeds but the rate of increase become smaller towards maturity.

The free soluble pectin increases and the other three pectic constituents decrease as the age advances and as the rotting sets in.

The down-grade changes begin to occur in storage conditions and they are responsible for the softening of the tissues which is caused by the separation of the cells due to loss of insoluble protopectin and middle lamella pectin from the cell walls. Bacteria and other organisms get a footing as it were when the tissues soften and hasten the rotting of the tissues.

The study of the effect of temperature on the pectic changes in mature tubers shows that the down-grade changes are much accelerated as compared to the similar changes in tubers stored at room temperatures.

All tubers pass through the same sequence of pectic changes which during the hot weather are accelerated like all chemical reactions.

The practical side of the potato storage is discussed in view of the results obtained and the lines on which further investigation should be undertaken are indicated.

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AGRICULTURAL METEOROLOGY.

STUDIES IN MICRO-CLIMATOLOGY, PART I.

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INTRODUCTION.

The study of weather in relation to agriculture is receiving increasing attention in many countries. The International Commission on Agricultural Meteorology as well as the Conference of Empire Meteorologists [1929] emphasized the importance of micro-climatology in relation to crops and the need for the study of "Local" or "Micro" climates and for the standardisation of apparatus and the methods of observation suitable for such studies. In usual meteorological practice, where weather-forecasting is the main object, one deals with observations of air temperature and humidity four feet above ground with the help of instruments kept inside a Stevenson screen situated in an open space. The detailed studies made in Europe by workers like Schmidt, Geiger [1930] and others have shown that the large scale eddies and the mixing brought about by them start at a height of about four feet above ground. One may therefore assume that at this level, the instruments are more or less outside the direct influence of the ground and the air layers near it.

In agricultural meteorology, however, it is precisely the layers that are usually avoided by the meteorologist which assume great importance. We are here concerned with the actual environment of the crops, *i.e.*, with "micro-climate" as distinct from "macro-climate". It is therefore necessary to elucidate questions like the following:—

(1) What is the relation between meteorological observations recorded at a standard observatory *A* where the exposure is unaffected by vegetation and those recorded at a similar station *B* situated in a small open space surrounded by crops?

(2) What is the relation between observations of temperature and humidity recorded at the station *B* and those recorded inside the surrounding crops ?

(3) How do the meteorological elements vary with height above ground inside and outside crops ?

(4) How do the meteorological elements vary with time during the day and during the seasons inside and outside crops ?

(5) How does micro-climate vary with the nature and density of plant population ?

(6) How does vegetation react on soil temperature and soil moisture ?

With a view to investigate some of these fundamental questions a meteorological station *B* in the farm of the Agricultural College, Poona, was started by the Agricultural Meteorology Branch of the Meteorological Office. The observations recorded at and around this station could be studied along with those recorded at the Observatory of the Meteorological Office which is situated at a distance of about a mile to the south-east of the Agricultural College. Regular observations were commenced at the station *B* from December 1932. Besides the usual observations, special observations of temperature and humidity were taken daily at the epochs of maximum and minimum temperatures both in the open site of the observatory and inside a few selected crops at short intervals of height up to 6 feet and later up to 10 feet above ground. An Assmann Psychrometer of the smaller type was used in taking these measurements.

During the first half of December 1932, comparative observations were made in the open, inside a mature '*kharif*' *jowar* field near the observatory *B* and inside a sugarcane field at a little distance from it. The '*kharif*' *jowar* was harvested on the 15th December. Later on observations were taken inside a few '*rabi*' crops in addition to those in the open and inside the sugarcane crop. These observations representing the conditions during the cool season when the climate is of the "dry continental" type will be discussed in the present series. The first part will deal with the observations made during the first half of December and a few points of general interest. In the later parts we shall discuss the micro-climates of a few crops, their statistical relationships and some interesting points brought out by hourly observations of air temperature, humidity and soil temperature, taken on selected days. Observations of soil moisture and soil temperature in relation to weather as well as the effect of crops on these factors will be discussed in another series of notes.

II. Methods of observation.

In order to record observations of air temperature and humidity many arrangements are possible. The important considerations are (1) cost of equipment, (2)

ability to take a series of observations in the least possible time and at a number of places, (3) reliability of the measurements.

To obtain a correct estimate of the actual air temperature and humidity at a particular point it is first of all necessary to screen the measuring instruments as effectively as possible from solar radiation as well as the radiation of bodies which are at a different temperature. The effect of disturbing influences can be reduced considerably by ventilating the sensitive parts of thermometer with a current of air of sufficient strength. An account of the various types of sheds or screens that may be used for housing a set of dry bulb, wet bulb, maximum and minimum thermometers will be found in the *Memoirs of the India Meteorological Department*, Vol. XXIV, Part III, "On Exposures of Thermometers in India". For the type of observations at small height intervals that we have in view the installation of a series of Stevenson screens of suitable size will be found very cumbersome, but their use cannot be totally dispensed with if it is desired to instal maximum and minimum thermometers or self-recording instruments at a few selected levels.

One efficient alternative for recording such observations accurately involves the use of a series of thermo-couples fixed on a post and a sensitive galvanometer housed in a small hut. The thermo-couples have to be provided with special shields and a series of these couples at small height intervals can be connected in a suitable manner to a common switch board. The same installation can be utilised for the measurement of soil and plant temperatures as well. A full description of this method and some results obtained with the installation in station *B* will appear elsewhere. The method, while it has its advantages, is not very convenient for field to field observations unless a costly portable galvanometer is used.

A third instrument suitable for field observations of temperature and humidity which is in extensive use is the whirling hygrometer. Being simple in construction it is comparatively inexpensive. It is useful in the open, if observations are required only at height intervals large compared to the length of the whirling element to which the thermometers are fixed. It is however somewhat difficult to manipulate, especially when a large number of readings are required inside crops, and is subject to radiation errors.

For micro-climatological observations the aspirated Assmann Psychrometer (R. Fuess) is the most satisfactory of the instruments so far evolved. The smaller type is better adapted to micro-climatic observations. The thermometers are shielded from radiation, and a ventilating mechanism causes a steady current of air to flow past the thermometer bulbs. The readings are reproducible under steady conditions and the instrument is sufficiently handy for observations inside the thickest foliage. On the whole this instrument is to be strongly recommended until

instruments of still smaller size and working on the same principles are evolved. A full description of the Assmann Psychrometer and the method of using it will be given in the ' Agricultural Meteorology Hand Book ' which is in course of preparation for the use of agricultural workers in India.

III. Observations of air temperature and humidity in the open air.

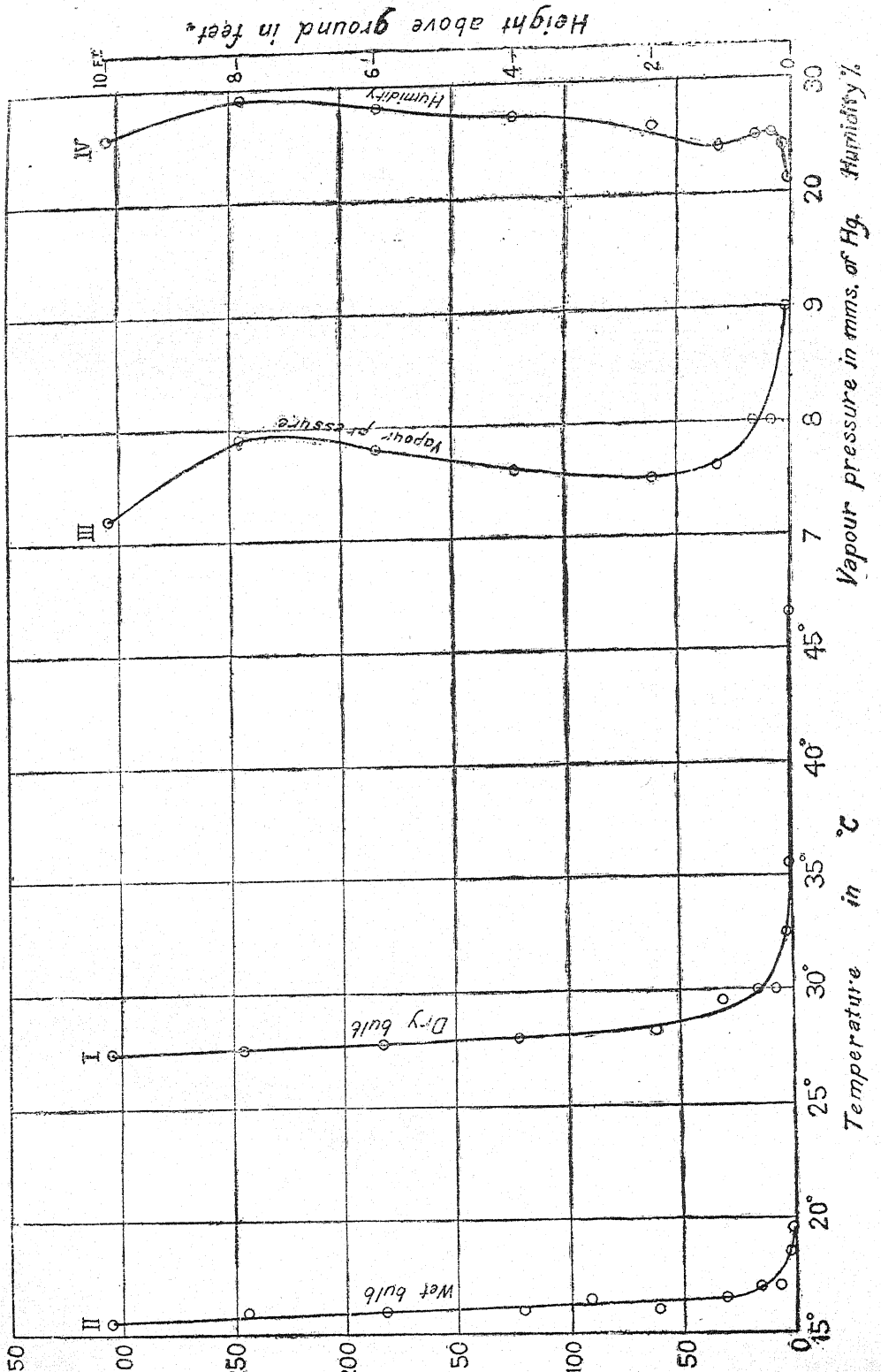
Maximum temperature epoch.

The fact that near the surface of the ground very high temperatures are observed at the maximum temperature epoch and that the temperature at different heights above ground decreases rapidly has been observed by those who have sought for an explanation of the inferior mirages that are usually associated with such temperature régimes near the ground. In this connection the observations made by Geiger [1930], Futi [1931], Malurkar [1931] and Ramdas [1932] are of special interest.

Fig. 1 shows a typical example of the variation of air temperature and humidity with height as observed in the open at the Agricultural Meteorological Observatory on 4th January 1933 at 13-00 hrs. I. S. T. In Fig. 1, curves I, II, III and IV show the dry bulb temperature, the wet bulb temperature, vapour pressure and percentage humidity respectively. It will be noticed that within a layer of about 10 cms. above ground, the dry bulb temperature falls rapidly by about 17 or 18° C. the fall being most rapid nearest to the ground. As compared to this variation, the variation higher up is small. The variation near the ground of the wet bulb temperature is less conspicuous than that of the dry bulb temperature. Vapour pressure is highest near the ground and decreases to smaller values higher up. The higher vapour pressure near the ground is mostly due to the evaporation of the moisture from the soil under the influence of solar insolation. Curve IV shows that the air higher up is slightly more saturated than near the ground.

We have just received a copy of the proceedings of the meeting of the International Commission of Agricultural Meteorology held at Munich (September 1932.)

Trankevitch (Vladivostok) gives conclusions somewhat similar to ours based on a study of the Phyto-climate of a wheat field and mentions that further work on these lines is in progress. The publication contains abstracts of a large number of papers from workers in various countries devoted to micro-climatology.



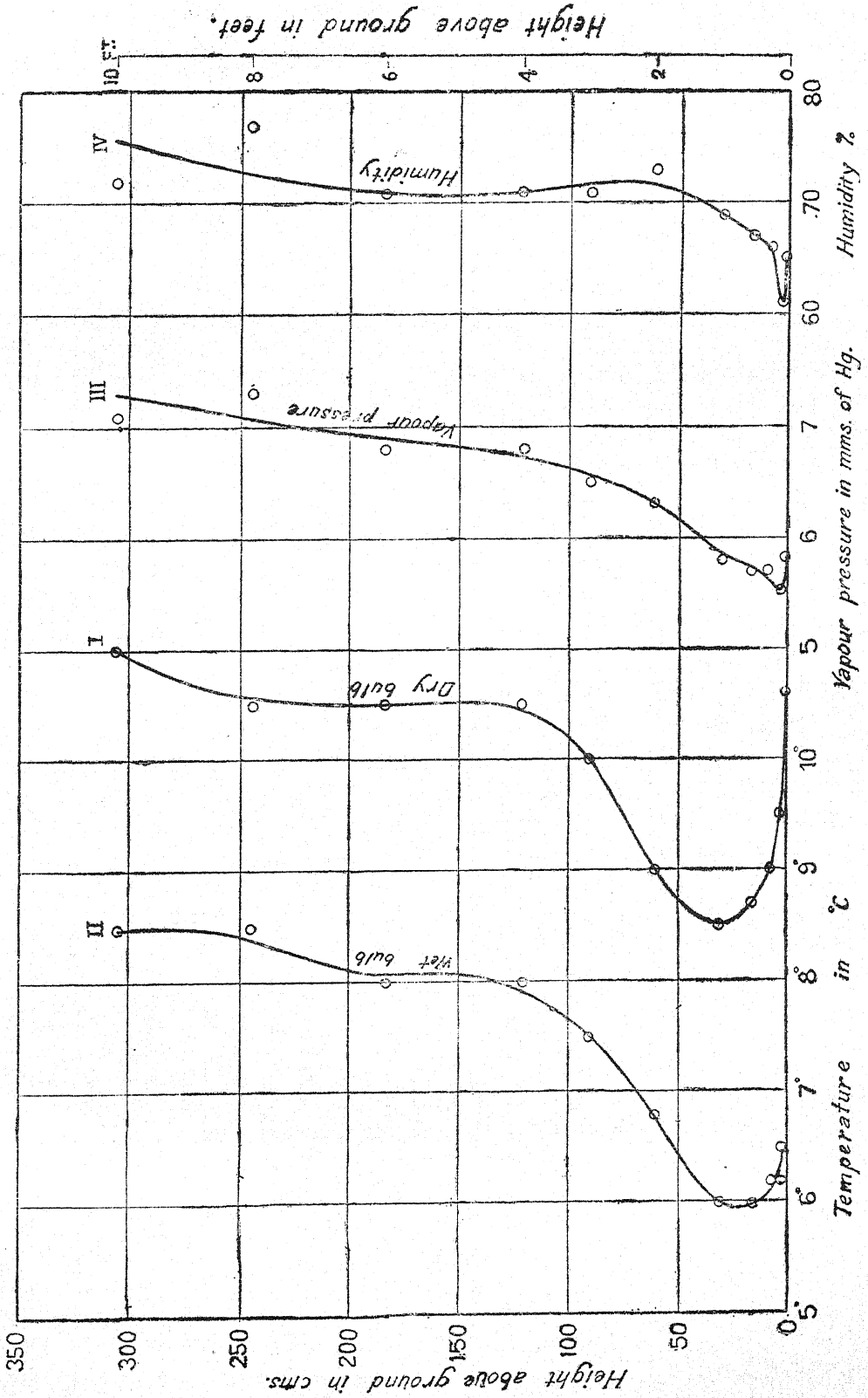
MAXIMUM TEMPERATURE EPOCH.

13·00 Hrs. I. S. T. on 4-1-1933.

Fig. 1. Variation with height of 'dry bulb' and 'wet bulb' temperatures, vapour pressure and humidity at Poona (Agricultural Meteorological Observatory) in the "open" on 4-1-1933.
"Dry Continental Type".

Minimum temperature epoch.

Fig. 2 shows the variation of temperature and humidity with height in the open at 5·00 hours I. S. T. on the 5th January. As in Fig. 1, curves I, II, III and IV refer to the dry bulb temperature, wet bulb temperature, vapour pressure and the percentage humidity respectively. Curve I shows that, as shown by Ramdas and Atmanathan [1932] the nocturnal inversion of temperature does not start right from the ground ; in the present instance it starts about 25 cm. above ground. Within the first 25 cm. there is a rapid fall of temperature with height though less conspicuously than in Fig. 1. It is remarkable that the lowest temperature during the night occurs not at the surface of the ground itself but at a distance above it. These features are shown also by the wet bulb temperature (curve II). Another interesting observation is that the pressure of water vapour which is seen to decrease with height during day shows an opposite tendency during the night (curve III). The percentage humidity curve (curve IV) indicates that the air is less saturated near the ground than higher up, a feature which is also seen in Fig. 1. The results discussed above are more or less of daily occurrence during the cool season and their explanation from physical considerations is being attempted in another paper.



MINIMUM TEMPERATURE EPOCH.

5·00 Hrs. I. S. T. on 5-1-1933.

"open" on 5-1-1933.

"Dry Continental Type".

Fig. 2. Variation with height of 'dry bulb' and 'wet bulb' temperatures, vapour pressure and humidity at Poona (Agricultural Meteorological Observatory) in the

IV. Observations in crops.

In this section we shall describe the observations taken during the first half of December 1932, from the 4th to the 15th, (*a*) in the open space of the observatory *B*, (*b*) inside a mature '*kharif*' *jowar* crop to the north of the observatory and (*c*) inside a sugarcane crop at a distance of about two furlongs to the east of the observatory.

The *jowar* field had an area of $\frac{1}{4}$ acre. The average height of the crop was 8 to 10 feet. The seed rate was 8 lbs. per acre and on an average there were about 10 plants per square yard. The variety used was 'Gidgap' and was sown for grain. The general condition of the crop was good. The grain was nearly dry. The date of sowing was 19th July 1932, the date of flowering 21st October 1932 and the date of harvest 15th December 1932.

The sugarcane (variety 'khadya') field had an area of one acre. The average height of the crop was 8 ft. 6 in. Distance between ridges (rows) was 4 ft., and eyebuds were 3 in. apart. The plant density was roughly six plants to a square yard, each plant having on an average four tillers. The date of planting was 1st February 1932, the date of germination 17th February 1932, the date of earthing 10th July 1932, the date of arrowing 30th November 1932 and the date of harvesting 3rd January 1933. The plot was irrigated periodically, the dates of irrigation being the 8th and 12th during the period under consideration.

The observations were taken at surface (·32 in.=0·8 cm.), 1 in. (2·54 cm.), 3 in. (7·62 cm.), 6 in. (15·2 cm.), 1 ft. (30·5 cm.), 2 ft. (61·0 cm.), 3 ft. (91·4 cm.), 4 ft. (122 cm.), and 6 ft. (183 cm.) above ground from a chosen spot at the centre of the field. The exact heights at which observations may be taken by agricultural stations will be discussed at the end of this series of papers. A graduated rod with the standard heights of observations marked on it was used for reference. The time spent by a trained observer in taking these observations at the maximum and minimum temperature epochs does not exceed an hour daily.

Maximum temperature epoch.

Table I gives the mean dry bulb temperature, wet bulb temperature, pressure of water vapour and the degree of saturation or percentage humidity during the period 4th to 15th December in the open, inside the *jowar* crop and inside the sugarcane crop respectively.

TABLE I.

Mean dry bulb and wet bulb temperatures, vapour pressure and humidity percentage at different heights in the "open" and inside "jowar" and "sugarcane" fields during the period 4th—15th December 1932, at the maximum temperature epoch.

"Dry continental type".

Height		Dry bulb temperature in °C.			Wet bulb temperature in °C.			Vapour pressure in mm. of mercury			Humidity percentage		
Inches	Centimetres	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane
0·3 in.	0·8	36·7	29·3	22·7	20·9	19·2	18·9	10·7	11·7	14·4	22·2	37·9	70·2
1·0 in.	2·54	34·8	29·3	23·0	19·9	18·5	18·6	10·0	10·7	13·9	23·7	34·9	66·3
3·0 in.	7·62	33·6	29·4	23·3	19·3	18·0	18·4	9·6	9·9	13·4	24·3	31·8	62·5
6·0 in.	15·20	32·5	29·7	23·8	18·7	18·0	18·1	9·2	9·6	12·7	25·3	30·4	57·7
1 ft.	30·50	31·6	29·9	24·6	18·3	17·8	17·7	9·1	9·2	11·8	26·0	28·7	51·4
2 ft.	61·00	31·0	29·7	25·5	17·7	17·5	17·4	8·5	8·8	10·7	25·3	28·2	44·1
3 ft.	91·40	30·6	29·8	26·4	17·5	17·5	17·1	8·5	8·8	9·9	25·8	28·1	38·6
4 ft.	122·00	30·0	29·9	27·0	17·0	17·1	16·9	8·5	8·5	9·4	26·7	26·7	35·4
6 ft.	183·00	29·8	30·0	27·5	17·2	17·3	16·8	8·5	8·5	9·0	27·1	26·7	32·8

The dry bulb temperature, vapour pressure and humidity per cent. are plotted in Fig. 3. As in Fig. 1, the dry bulb temperature in the open is very high near the ground and falls rapidly with height. The temperature inside the *jowar* crop is lower than in the open and is fairly constant at all heights up to which observations were recorded, except near the ground where it is slightly less. In the sugarcane crop, owing to the wet nature of the soil, the temperatures are very much lower than in *jowar*. The temperature is lowest near the soil and increases with height rapidly, but even at 180 cm. it is about 2·5°C. lower than in the open. The largest difference between "open" and "sugarcane" temperatures is 14°C. near the soil; the difference is 5·5°C. at 61 cm. and 3°C. at 122 cm. The cooling near the soil is of course brought about by the large amount of evaporation.

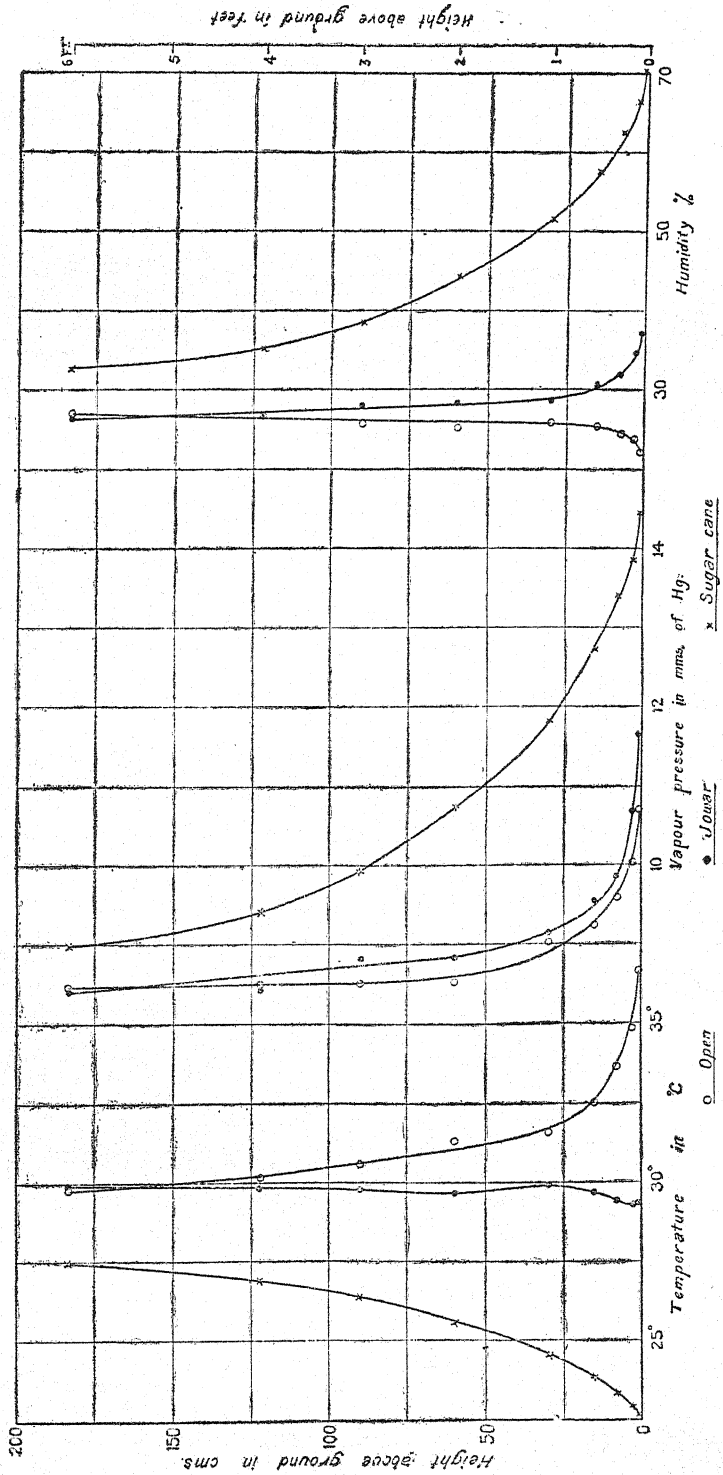


Fig. 3. Variation with height of 'dry bulb' temperature, vapour pressure and humidity at Poona (Agricultural Meteorological Observatory) inside and outside crops during the period 4th—15th December 1932.
"Dry Continental Type".

This is very clearly brought out by the curves showing the variation of pressure of water vapour. It will be seen that the vapour pressure in the *jowar* is only slightly more than in the open whereas in the sugarcane field it is considerably higher than in both the other cases and at all heights. In all the three curves of vapour pressure, the tendency is for the values to decrease rapidly with height, as in all cases evaporation from the soil goes on during the day and there is mixing with the drier air at higher levels. The effect is, however, most conspicuous in the sugarcane field. The curves showing the variation of humidity percentage with height are equally interesting. In this case the degree of saturation increases with height in the open, and decreases with height inside the two crops, the falling away of the degree of saturation being most conspicuous in the sugarcane field.

It may be noted that the variations of the meteorological factors are very conspicuous during this period of the day both in the open as well as inside crops. The activity of plant life is also more pronounced at this time. Further, one is surprised to meet with variations of the same order in meteorological factors in a single day and at the same instant due to change of environment as one observes from season to season during a year in the open space at 4-ft. level. It is therefore very important to make a thorough study of micro-climate in order to fully appreciate the mutual dependence of weather and crops. One might say that, while the general "open air" weather has influence on plant life, the plants also profoundly modify the weather factors in their immediate neighbourhood. The limitations of the usual meteorological data accumulated at "macro-climatic" stations in their applications to plant growth and crop yields becomes very evident during a study of the micro-climate.

Minimum temperature epoch.

As may be anticipated, the variation of the micro-climate at this epoch should be much less than during the day. The weather factors at night also have an important bearing on the welfare of crops, *e. g.*, if the temperature becomes very low, there is the danger of frost, especially in North India.

One is therefore interested to know how the air temperature varies with height, where the lowest temperatures occur and how these conditions depend on the amount of moisture present in the air and how they vary from crop to crop. These are some of the problems of fundamental importance that await solution.

The mean dry bulb temperature, wet bulb temperature, vapour pressure and humidity percentage at the minimum temperature epoch in the 'open', inside *jowar* and inside sugarcane are given in Table II. These values, excepting those of the wet bulb temperature are plotted in Fig. 4.

TABLE II.

Mean dry bulb and wet bulb temperatures, vapour pressure and humidity percentage at different heights in the "open" and inside "jowar" and "sugarcane" fields during the period 4th—15th December 1932, at the minimum temperature epoch.

"Dry continental type."

Height		Dry bulb temperature in °C.			Wet bulb temperature in °C.			Vapour pressure in mm. of mercury			Humidity percentage		
Inches	Centi-metres	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane
0·3 in.	0·8	10·5	12·1	12·1	9·4	10·9	11·6	8·3	9·2	10·1	86·4	86·7	95·2
1·0 in.	2·54	10·2	11·6	11·7	9·3	10·7	11·2	8·4	9·2	9·7	89·2	90·1	94·7
3·0 in.	7·62	9·9	11·3	11·3	9·1	10·3	10·8	8·3	9·1	9·5	90·4	91·0	94·3
6·0 in.	15·20	9·8	10·9	11·0	9·1	10·2	10·5	8·4	9·1	9·3	91·5	92·7	94·5
1 ft.	30·50	10·0	10·7	10·7	9·4	10·2	10·3	8·6	9·0	9·2	92·7	93·0	94·7
2 ft.	61·00	10·2	10·6	10·7	9·5	10·1	10·3	8·7	9·0	9·2	93·1	93·7	94·8
3 ft.	91·40	10·4	10·5	10·8	9·7	10·0	10·4	8·8	9·0	9·3	92·3	94·3	94·8
4 ft.	122·00	10·5	10·5	10·9	9·9	10·1	10·5	8·9	9·1	9·3	92·3	95·3	94·3
6 ft.	183·00	10·8	10·7	11·0	10·1	10·3	10·5	9·0	9·3	9·3	92·1	96·3	94·3

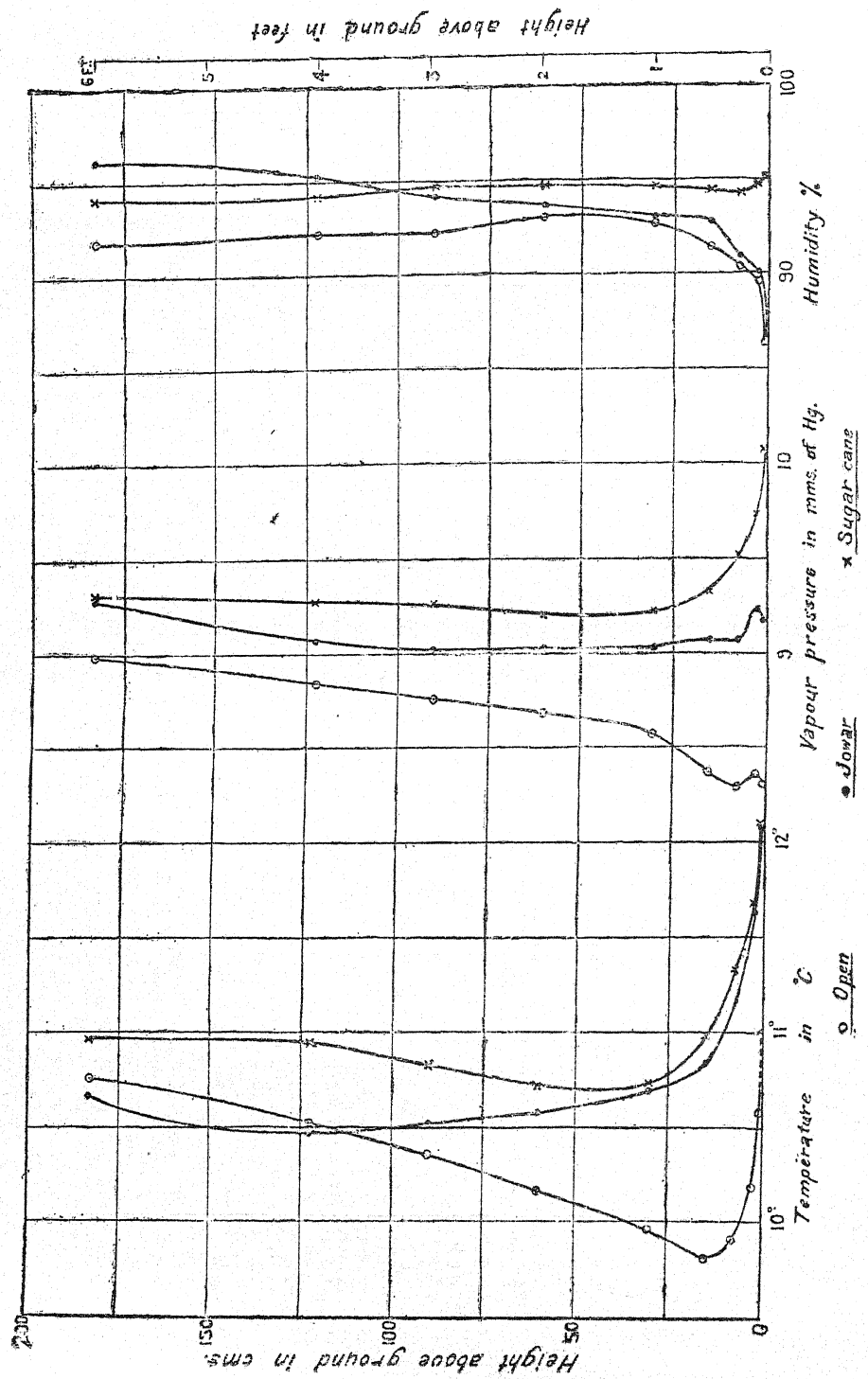


Fig. 4. Variation with height of 'dry bulb' temperature, vapour pressure and humidity at Poona (Agricultural Meteorological Observatory) inside and outside crops during the period 4th—15th December 1932.
". Dry Continental Type".

The temperature scale is magnified. In all the three curves the dry bulb temperature at first decreases with height and then begins to increase. The level of inversion is lowest in the open (15 cm.) and higher up in the sugarcane (about 45 cm.) and highest in the *jowar* (above 100 cm). It may be noted that the lowest temperatures are recorded at these inversion levels.

The fact that the lowest temperature at night occurs at some height above ground raises the questions whether other places are likely to behave similarly and whether it would not be possible to vary the intensity and position of this level by altering plant density and the water content of the upper layers of the soil. The thermal conductivity of the soil will of course be an important controlling factor. The actual temperatures inside the crops are higher than in the open, the air in the sugarcane crop being warmer at all levels whereas, inside *jowar*, the temperature tends to the open air value above 100 cm.

The vapour pressure in the open increases with height but is throughout less than inside the crops; it is fairly uniform in *jowar* except above 125 cm. where there is a rising tendency; in the sugarcane it decreases with height in the lower layers (as during the day) but the variation is negligible above 50 or 60 cm. The vapour pressure inside sugarcane continues to be higher than in the 'open' and in *jowar* at this epoch also. The percentage humidity curves show that in the open and in *jowar* the air is least saturated near the soil and that the degree of saturation increases with height; this rising tendency is limited in the open to 50 or 60 cm. whereas in the *jowar* it persists at all heights. The air in the sugarcane crop is most saturated near the soil and, after a slight fall, remains steady at other levels.

Range or difference between observations at maximum and minimum temperature epochs.

Table III gives the difference between the corresponding values of Tables I and II. As the observations were taken during two periods which represent the steady conditions prevailing at the daily maximum and minimum epochs of temperature the "range" given in Table III represents the diurnal range.

TABLE III.

The mean diurnal range of dry bulb and wet bulb temperatures, vapour pressure and humidity at different heights in the "open" and inside "jowar" and "sugarcane" fields during the period 4th—15th December 1932.

Height		Dry bulb temperature			Wet bulb temperature			Vapour pressure			Humidity percentage		
Inches	Centi-metres	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane	Open	*Jowar*	Sugarcane
0·3 in.	0·8	26·1	17·2	10·6	11·5	8·3	7·2	2·4	2·5	4·3	64·3	48·8	25·0
1·0 in.	2·54	24·6	17·7	11·3	10·7	7·9	7·4	1·7	1·4	4·1	65·4	55·2	28·4
3·0 in.	7·62	23·7	18·2	12·0	10·1	7·7	7·6	1·2	0·8	3·9	66·1	59·2	31·8
6·0 in.	15·20	22·7	18·9	12·8	9·5	7·7	7·6	0·9	0·5	3·4	66·2	62·3	36·7
1 ft.	30·50	21·6	19·2	13·8	8·9	7·6	7·5	0·6	0·1	2·6	66·7	64·3	43·3
2 ft.	61·00	20·9	19·2	14·8	8·2	7·4	7·1	—0·1	—0·2	1·5	67·8	65·6	50·7
3 ft.	91·40	20·2	19·3	15·6	7·8	7·5	6·7	—0·3	—0·2	0·7	66·5	74·2	56·3
4 ft.	122·00	19·5	19·4	16·0	7·5	7·2	6·5	—0·3	—0·6	0·1	65·6	68·5	58·8
6 ft.	183·00	19·0	19·3	16·5	7·1	6·9	6·3	—0·5	—0·8	—0·3	65·0	69·7	61·5

Dry bulb temperature.

In the open the range of dry bulb temperature is 26·1 °C. at 0·8 cm. and decreases rapidly to 19·0°C. at 183 cm. ; in *jowar* and sugarcane the range is a minimum near the soil and increases with height. The least variation during 24 hours occurs inside sugarcane.

Wet bulb temperature.

In the ' open ' and '*jowar* ' the diurnal range decreases with height; in sugarcane there is a ' maximum ' value at about 15 cm.

Vapour pressure.

In all the three cases the range is a maximum near the soil; in the open and *jowar* there is a reversal of sign at about 60 cm. above which the vapour pressure is greater at night than by day. This reversal is seen to occur at a higher level in the case of sugarcane.

Humidity.

In the open the range of humidity is uniform at all levels; in *jowar* and sugarcane the range increases with height.

In conclusion we wish to record our best thanks to the Principal of the Agricultural College, Poona, for giving the necessary facilities for this work in the College Farm and to the Director-General of Observatories, Meteorological Office, Poona, for giving us the loan of the equipment for observational work.

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THE LANTANA SEEDFLY IN INDIA, *AGROMYZA (OPHIOMYIA) LANTANAE* FROGGATT.

BY

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(Received for publication on 2nd October 1933)

(With Plate XXXI.)

It is well known that the plant *Lantana camara* Linn., introduced into India as an ornamental plant years ago, has spread far and wide throughout the country and become a very noxious pest overrunning uncultivated waste lands, grazing grounds and forest lands. The question of eradication of the plant has been engaging the attention of the Government for the past many years.

Successful control of this plant in Hawaii by the introduction of a seedfly from Mexico, the original home of the plant, about 33 years ago and afterwards into Fiji, New-Caledonia and Queensland had been reported.

A special officer, Rao Sahib Y. Ramachandra Rao, (then Assistant Entomologist, Madras Department of Agriculture) was appointed by the Government of India 16 years ago to enquire into the efficiency of indigenous insects as a check on the spread of lantana in India and an insect survey of the plant was made by this officer all over India and Burma for over two years. In his interesting report (*Mem. Dept. Agric., India, Ent. series,* **5**, No. 6, 1920, p. 279) containing the results of his tours, he has come to the conclusion that any insect of the nature of the lantana seedfly is absent in India. In coming to this conclusion he states that " if such an insect were really present in India, the fact would, irrespective of the time of visit, easily have forced itself on my attention, for, even if it did not occur throughout the year, sufficient traces of its presence were bound to have been noticeable even in the most adverse part of the year. "

While the writer feels considerable hesitation in questioning a statement made by a specialist, he considers it his duty to give publicity to the following facts which go to prove the existence of the lantana seedfly in different parts of India.

Attempts to introduce the lantana seedfly from Hawaii by importing infested seeds have been made at five different times by the Mysore Department of Agriculture and although most of the insects were received dead in all cases, only in one

instance in 1921, a few flies emerged and these were liberated in Bangalore. The weather conditions at the time of the release were very unfavourable, as heavy showers of rain followed the liberation of the flies. Careful search made for the recovery of the flies round about Bangalore did not reveal the presence of the flies breeding in the berries. The search was continued next year also without any result and it was presumed that the flies failed to breed, particularly in view of the very unfavourable weather conditions in which they had to be liberated.

Recently however the writer during his entomological duties, had to examine lantana fruits in Bangalore and was surprised to find the seedfly borer. The thought at once occurred to the writer that this might be the result of the 1921 liberation at Bangalore. In order to test this, parcels of lantana fruits were obtained from different and widely separated places in the State, *viz.*, Mysore, Chikmagalur, Saklespur and Mysore Coffee Experiment Station, Balehonnur (coffee areas), Government Farm, Marthur (Shimoga District, near the Western Ghats) and the infestation of the berries by the seedfly was found to range from 2 to 5 per cent. The infestation was noted to be more in the coffee areas than in other places.

To find out whether the insects are confined to Mysore State alone, small consignments of lantana fruits were obtained from Coimbatore, Cochin, Shencotta and Quilon (Ghat area and sea coast of Travancore) and Maymyo in Burma. Fruits from all the above places showed fly infestation and large numbers of flies emerged. During a very recent visit to North India and Bombay (August 1933) the writer was able to collect a large quantity of fly-infested berries from lantana bushes in the Botanical Gardens, Saharanpur (U. P.) and in the gardens attached to the Museum and to the statue of the Marquis of Wellesley, Bombay.

During a visit to Ceylon by the writer last April, a number of lantana fruits examined by him in Kolongasayya Estate (about 60 miles north of Colombo) showed the characteristic whitish streaks indicating the presence of fly maggots on the surface of the fairly matured berries indicating damage to fruits by fly maggots, but unfortunately no specimens were collected there.

As regards injury to the fruits in Hawaii, the late Dr. K. Kunhikannan, Entomologist, Mysore Department of Agriculture, who visited Hawaii in 1921 states that "the damage it did was more to the pulp of the fruit in which the egg was laid and the larvæ developed for pupation. The larva usually sought the pithy hollow to be found between the germ cells in every seed. It is obvious that all that is vital to the full grown seed is left untouched". (*Agricultural Journal of India*, Vol. XIX, part v, p. 505).

In the opinion of the entomologists in foreign countries the fly maggot is said to destroy the germinating power of the seeds in the growing berries. The observa-

tions made by the late Dr. K. Kunhikannan during his stay in Hawaii do not evidently agree with the authoritative observations of the foreign entomologists. Whether the germinating power of the infested seeds is affected or not is being investigated by the writer.

The writer has observed that in several instances, the fly maggots after developing for some time in the fleshy portion of the berries, bore into the fruit receptacle and complete their development there, without entering the seeds. This observation was made both in the infested material obtained in India and from Hawaii.

Whatever may be the differences observed so far in the habits of the seedfly in Hawaii and in India, it has been definitely found by experts both in the British Museum and in Hawaii,* that the fly reared out by the writer recently from infested lantana berries in different parts of India, is the species identical with the well known Hawaiian fly *Agromyza* (*Ophiomyia*) *lantanae* Froggatt.

It is a great temptation to say that the few Hawaiian flies liberated in Bangalore 12 years ago have spread to Travancore, Bombay, Saharanpur, Burma and perhaps to Ceylon also. The writer thinks that it is unlikely that these few flies have spread far and wide, but that the fly has been present in different parts of India for a long time, perhaps ever since the introduction of lantana into India. It must have escaped the notice of the Mysore Entomologist when he was examining the lantana fruits for the recovery of the flies liberated in Bangalore and of the special lantana insect survey officer appointed by the Government of India. As to why the fly has not been an effective agent in India in keeping down lantana has to be further investigated. No parasites have as yet been bred from infested material that has been collected.

In conclusion the writer wishes to add that the main object of preparing this paper for publication is to bring to the notice of people interested in the subject, the fact that the well known lantana fly of Hawaii is at present widely distributed in different parts of India. Now that it has been discovered, investigations on the ecology of the insects under Indian conditions and other points are being made by the writer.

* The lantana seed flies reared out from lantana fruits in India sent to Dr. Swezey, Consulting Entomologist, Hawaiian Sugar Planters' Association, for identification, have been received identified as *Ophiomyia lantanae* Froggatt, the same species of fly that is known to keep lantana in check in foreign countries. The generic name *Agromyza* has lately been changed to *Ophiomyia* by Bezzi in his 'Diptera of Fiji Islands'.

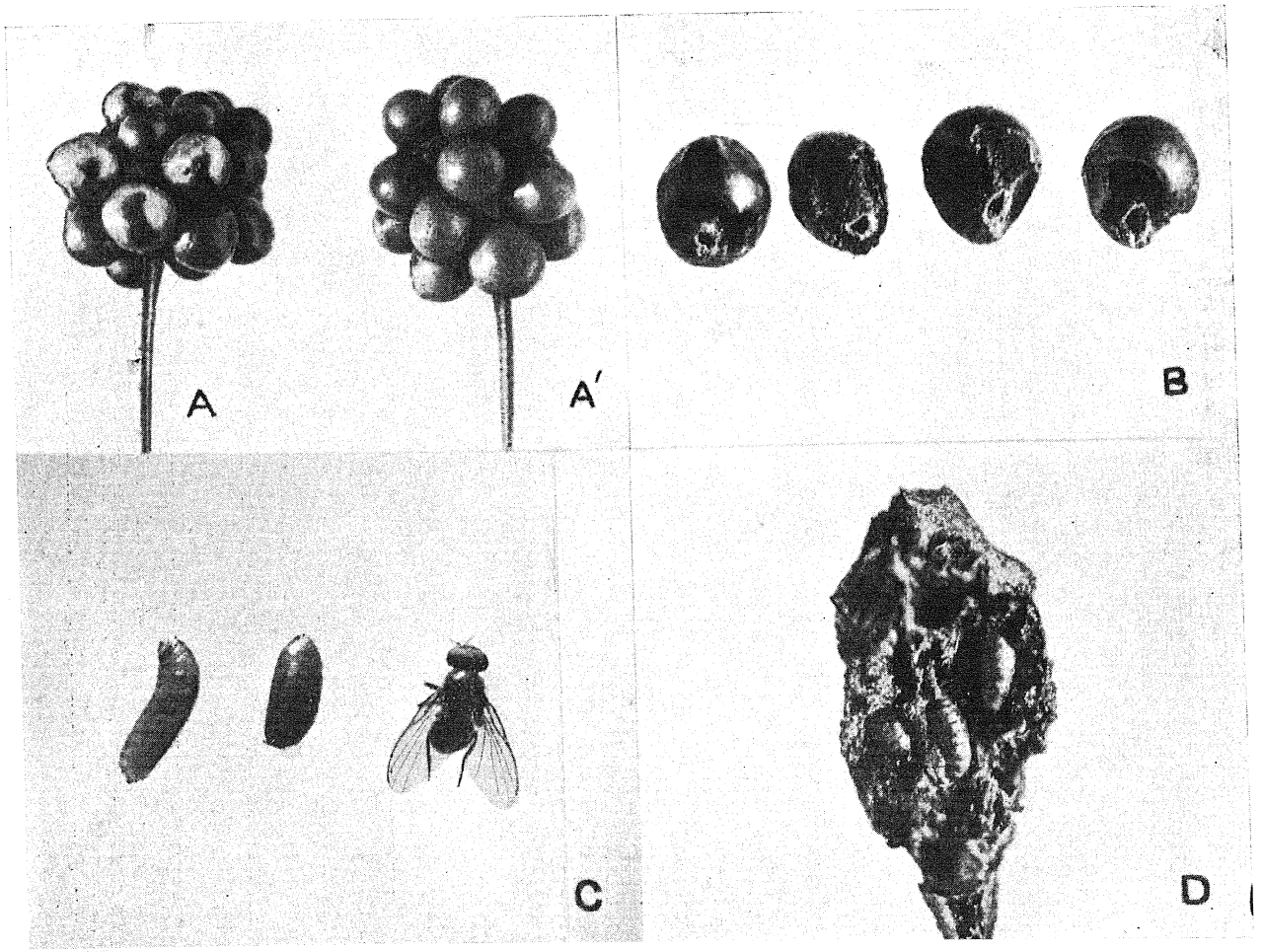


Fig. A. Unripe lantana fruit cluster with five berries showing the characteristic whitish appearance on the fruit surface produced by the maggots tunnelling and damaging the pulp.

Fig. A'. Unripe lantana fruit cluster with healthy berries.

Fig. B. Lantana fruits showing the holes on the seeds made by the maggots entering the pithy hollow inside.

Fig. C. The adult fly, pupa and maggot.

Fig. D. The fleshy fruit receptacle cut open to show the pupae of the fly and the tunnels and cavities made by the maggots.

PLATE XXXI.

A NOTE ON THE DIFFERENTIATION OF HAIRS FROM THE EPIDERMIS OF COTTON SEEDS.

BY

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(Received for publication on 12th October 1932)

(With Plates XXXII-XXXIV.)

The object of the present note is to adduce photomicrographic evidence in favour of continuous differentiation of hairs on growing cotton seeds; and to reply to certain points raised in criticism of the writer's first paper [Gulati, 1930] by Barritt [1932] who called for photomicrographs to substantiate the camera lucida drawings already published.

Since the appearance of Barritt's criticism, the cytology of the epidermal layer of growing cotton seeds has been re-studied from five representative types of Indian, American, and Egyptian cottons. These cottons are :—

1. *Gossypium sanguineum* × *cernuum* . . .	Indian types.
2. *G. indicum Mollisoni*	
3. *G. hirsutum* Punjab-American 289 F. . .	American type.
4. *G. barbadense* Ashmouni 37	Egyptian types.
5. *G. barbadense* Boss III	

The material of the first three cottons was collected by the writer, while that of the remaining two was kindly supplied by Mr. K. I. Thadani, Botanist in Sind (India).

The results of this repetition of observations are in perfect agreement with the writer's earlier conclusions and are described below briefly.

Cytological observations.

These observations bring out three points namely :—(1) Mitotic division of epidermal cells does not cease after the first day of flowering, and is shown to exist in longitudinal sections of seeds up to the tenth day of their development after flowering. (2) Hair cells appear to go on sprouting on the growing seed till about the third week. Hairs emerging from the epidermis are shown in close proximity to old ones in sections of seeds up to the tenth day again. (3) Microtome sectioning

of growing cotton seeds is not impossible as is categorically affirmed by Barritt. Whereas the presence of mitotic division in epidermal cells affords definite proof of their multiplication in number with increasing age, the presence of new hairs near old ones remains suggestive of their continuous differentiation. Photomicrographs illustrating these points are arranged in three plates.

Plate XXXII shows that complete and satisfactory sections of cotton seeds, up to the third week of development, are possible to prepare by the usual paraffin technique [Lee, 1928]. The fixatives employed were :—(1) Flemming's weaker formula of aceto-osmo-chromic acid, and (2) Bouin's aceto-picro-formol. The staining of sections was done by triple staining technique, involving the use of safranin, gentian violet, and orange G, where Flemming's fixative was used ; and Mayer's Haemalum and eosin when Bouin's fluid was employed.

Plate XXXIII shows the occurrence of mitotic division in epidermal cells. Except in the case of Fig. 7, which shows the metaphase stage of division, it is the telophase stage shown in all the other figures. Regarding Barritt's and even Ayyar and Ayyangar's [1933] failure to observe actual mitosis after the second day there can be two possible reasons. The first reason is that they examined transverse sections only as also stated by them. The writer also found it extremely difficult to detect a complete section of the dividing nucleus in transverse sections. All the mitotic stages, figured here, are from longitudinal sections. In all likelihood, the difficulty is due to the plane of mitotic spindle lying along the longitudinal axis of the seed during this early period of growth. The second reason is that the pigment inclusions of epidermal cells mask these structures even when they are present. In order to get over the second difficulty, the use of Bouin's fixative is recommended in preference to the Flemming's fluid.

Plate XXXIV shows "new" hairs near the bases of old ones in 4th to 10th day seeds. The somewhat regular outgrowth of hair cells on the first day in Fig. 1, and quite a number of "new" hairs in Figs. 3 and 6 from 7th and 10th day seeds are noteworthy features of this plate.

The bulk of these photographs, as will be noticed from the legends, are from *Gossypium sanguineum* × *cernuum* while the other four cottons mentioned above are only represented by one or two figures for each of them. The reason for giving such a preference to *G. sanguineum* × *cernuum* was that the camera lucida drawings published earlier were from the material of this cotton.

The other points raised by Barritt [1932] and grouped as statistical and genetical objections are answered below.

Statistical objections.

These objections refer to the writer's data of cell and hair counts and are based on (1) fragility of hair cells in early stages ; (2) unrecognisable state of

some hair cells on the first day ; (3) variability of the length of tangential axis of epidermal cells in relation to their plane of sectioning, and (4) relationship between the surface area of seed and an average epidermal cell.

(1) The fragility of cells noticeable in defective microtome sections was not operative in the writer's counts as they were made from free hand sections of freshly obtained material. The boll as a whole can be easily handled and cut into sections of seeds with an ordinary razor. The varying thickness of hand sections is however likely to cause some error. This was curtailed to the minimum by counting only in one plane of focus at a place under similar magnification, while examining sections of seeds at different stages of growth.

(2) If all the hair cells are in existence on the first day and are not necessarily recognisable in surface view, as stated by Barritt, the only possibility for the " unseen " hair cells is to look like ordinary epidermal cells. The view of continuous differentiation of hairs also starts from such a state.

(3) The variability in the length of tangential axis of an epidermal cell is, indeed, great. But it is impossible for all the cells in the periphery of a median transverse section to present this length consistently too small or too large. In fact, if some cells get cut in narrow corners, others will have to be cut diagonally to make up the circumference of the median section. Thus, the mean value of the transverse axis obtained from a number of cells all round the periphery is not likely to be very far from a really dependable value. Barritt was possibly imagining the case of marginal cells in surface sections when this point occurred to him.

(4) Barritt found confirmation of his 33-fold increase in surface area of the 28th day seed, in Farr's [1931] value of 31·7-fold increase ; and, therefore, stated that an average epidermal cell at that age would be 33 times as big as an average cell on the first day. Actual measurements of surface area of ten epidermal cells from the middle of surface sections at various stages of seed development, however, show that the increase in mean surface area of an epidermal cell up to the 20th day is only 4-fold. The mean values obtained from 1st, 4th, 7th, 10th and 20th day cells were of the order of 26·4, 29·3, 29·4, 54·0, 106·1 respectively. It will be seen from these values that there is not much change in surface area of an average epidermal cell up to the 7th day. This absence of increase is in agreement with Farr's conclusion that up to the 12th day, extension of epidermal covering is chiefly through the division of these cells. Further, the writer's values of increase in transverse axis of epidermal cells in the 28th day seeds, such as 3·1-fold in Punjab-American 4F, 2·9-fold in Punjab-American 289F and 2·3 in N. 14, receive remarkable confirmation from Farr's [1931] 2-fold increase in cells of her corresponding material.

Genetical objection.

With reference to Barritt's objection that naked seeded cottons cannot be induced to develop fuzz under any system of cultivation, and that amount of fuzz is not subject to environmental conditions, attention is adverted to the work of Kearney and Harrison [1927, 1928]. They conclude that nakedness of seed being heterozygous in Upland cotton reverts to fuzzy seeds in Mendelian ratio; and that the position of bolls on upper or lower branches, near the main stem or away from it, is conducive to greater or less development of fuzz on the seeds. Ayyar and Iyengar [1932] have also found that the number of epidermal cells sprouting into hairs varies also with the position of the seed in a lock.

It may also be noted that further support to the view of continuous differentiation of hairs on growing cotton seed is afforded by Armstrong and Bennett's [1933] recent work.

Acknowledgment.

The writer has much pleasure in thanking Dr. Nazir Ahmad for his helpful suggestions in writing this note.

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Explanation of plates XXXII-XXXIV.

Plate XXXII.

Figures (1-4) show complete sections of cotton seeds at different stages of development.

Gossypium sanguineum × *cernuum.*

Fig. 1. Transverse section of 20th day seed (× 17·5).
Fig. 2. Transverse section of 7th day seed (× 33·5).
Fig. 3. Longitudinal section of 7th day seed (× 33·5).
Fig. 4. Transverse section of 1st day seed (×61).

PLATE XXXIV.



Fig. 1.

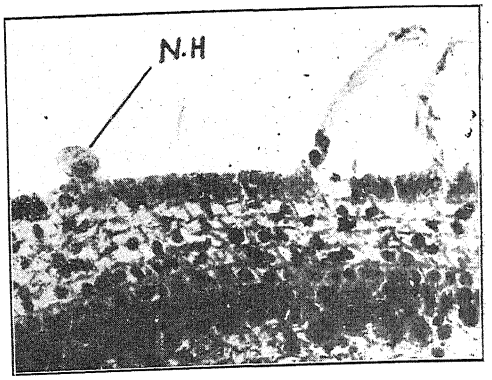


Fig. 2.

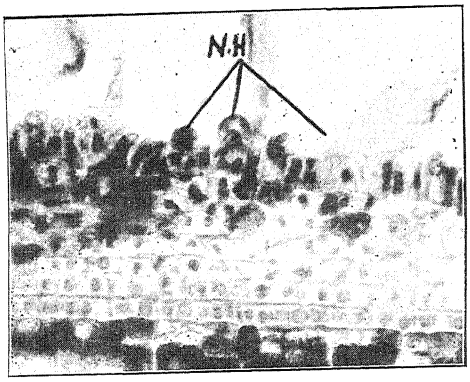


Fig. 3.

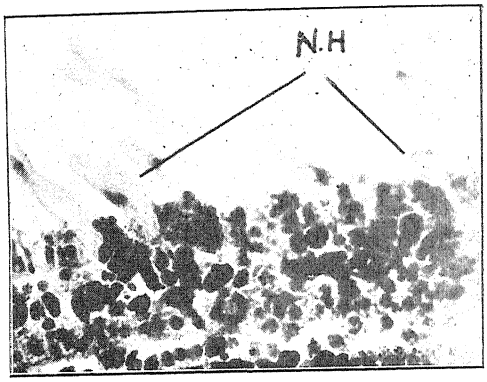


Fig. 4.



Fig. 5.

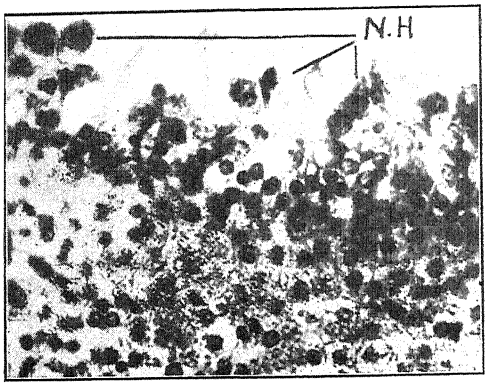
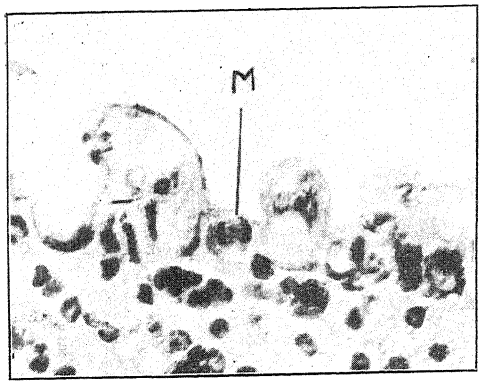


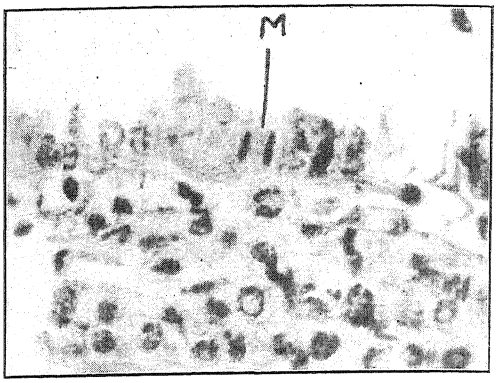
Fig. 6.

(*For explanation see page* 475.)

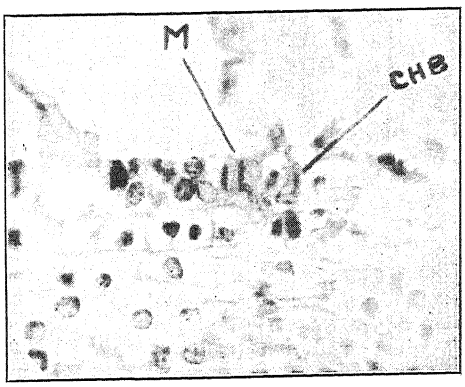
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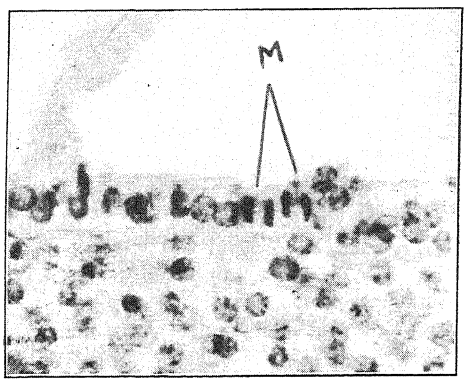
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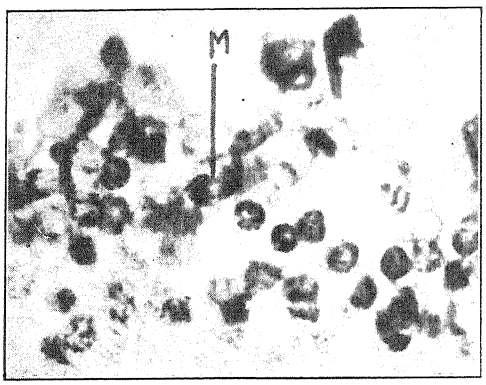
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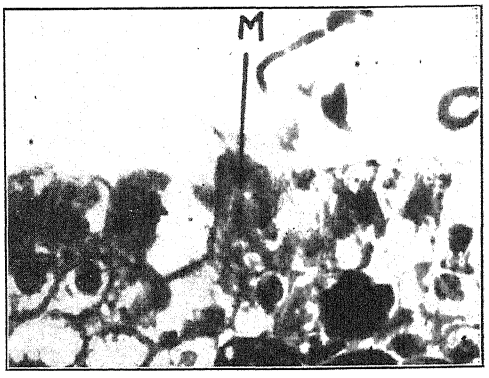
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(*For explanation see page 475.*)

PLATE XXXII.



Fig. 1.

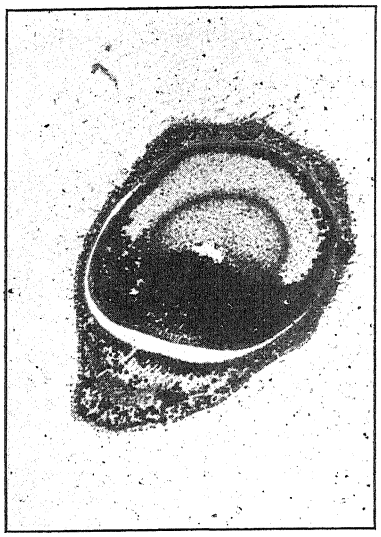


Fig. 2.

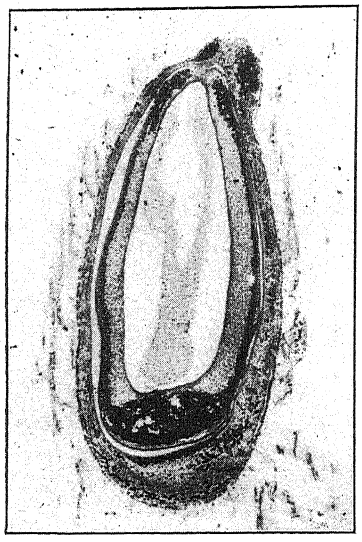


Fig. 3.

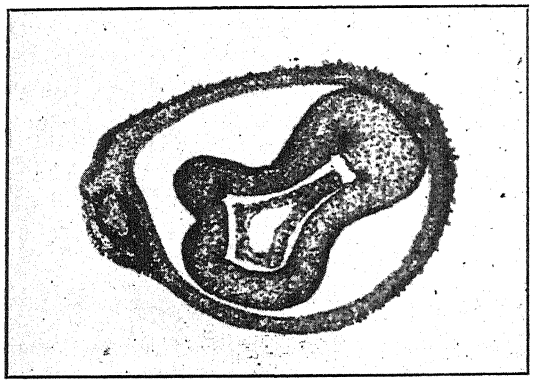


Fig. 4.

(*For explanation see page* 474.)

PLATE XXXIII.

Figures (1-8) show mitotic division in epidermal cells.

Gossypium sanguineum × *cernuum.*

Fig. 1. Longitudinal section of 1st day seed (×780).
Fig. 2. ,, ,, 4th ,, ,,
Fig. 3. ,, ,, 7th ,, (×600).

G. hirsutum 289 F. Punjab-American.

Fig. 4. Longitudinal section of 7th day seed (×780).

G. indicum Mollisoni. Punjab-Desi.

Fig. 5. Longitudinal section of 7th day seed (×780).
Fig. 6. ,, ,, 10th ,, ,,

G. barbadense.

Fig. 7. Longitudinal section of 7th day seed of Ashmouni 37 (×780).
Fig. 8. ,, ,, 10th ,, ,, Boss III (×780).

M stands for mitotic division.
C. H. B. stands for constricted hair base.

PLATE XXXIV.

Figures (1-6) show growth of new hairs in close proximity to old ones.

Gossypium sanguineum × *cernuum.*

Fig. 1. Longitudinal section of 1st day seed (×375).
Fig. 2. ,, ,, 4th ,, ,,
Fig. 3. ,, ,, 7th ,, ,,
Fig. 4. ,, ,, 10th ,, ,,

G. barbadense.

Fig. 5. Longitudinal section of 7th day seed of Ashmouni 37. (×375).

G indicum Mollisoni.

Fig. 6. Longitudinal section of 10th day seed showing quite a number of "new" hair outgrowths. (×375).

N. H. stands for "new" hair cells.

STUDIES ON THE HYDROLYSIS OF STARCH BY THE ENZYMES IN *CHOLAM* MALT, *SORGHUM VULGARE**.

BY

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(Received for publication on 15th August 1933.)

(With thirteen text-figures.)

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Introduction.

The subject of *cholam* malt has been engaging the attention of workers in India for several years past. Viswanath, Rao and Aiyangar [1918] first made a careful examination of several of the South Indian cereals with a view to select a suitable substitute for barley for malting operations and for the preparation of various malted foods, of which nearly rupees three million worth are being imported into India annually. They finally selected *cholam* as the most promising of such substitutes.

A more detailed study of the fitness of *cholam* for malting operations was made by Norris and Viswanath [1923], who compared 18 varieties of *cholam* and found that the best of them gave almost the same kind of extract as barley. They also examined the optimum conditions of steeping, couching and drying and made a detailed comparison of the action of *cholam* and barley malt extracts on starch as a result of which they noted the following important differences between them:—(1) *Cholam* malt appeared to possess a higher liquefying power than barley malt, as

* Abridged from a thesis approved by the University of Madras for the degree of Master of Science. The work was carried out under the guidance of Rao Bahadur B. Viswanath, F.I.C., F.C.S., Agricultural Chemist to the Government of Madras, to whom the author desires to express his grateful thanks.

shown by the change in iodine colouration, but a lower saccharifying capacity as measured by cupric reduction. (2) The saccharification curves for *cholam* malt were similar both at normal and at higher temperatures, while the curves for barley showed marked differences. (3) The deficiency in saccharifying power of *cholam* was apparent only in the earlier stages, since eventually, the sugar production from *cholam* caught up and even surpassed that from barley. These differences were explained on the basis of the two-enzyme theory, by supposing that *cholam* malt diastase contained more of the amylase (liquefier) than the dextrinase (saccharifier), and that the dextrinase in *cholam* is more stable at higher temperatures than the dextrinase of barley.

The above results were further confirmed by Viswanath and Suryanarayana [1925] who paid special attention to the mechanism of the action in the two cases and the nature of the products formed. The apparent "lag" of *cholam* in the earlier stages and its subsequent catch up of barley was explained on the supposition that in the case of *cholam*, dextrins of a lower order than with barley had to be formed before the dextrinase could act on them. The products of hydrolysis of starch by *cholam* malt, they found, consisted of a small quantity of maltose and comparatively larger amounts of dextrins of low rotation and high reducing power, whereas those resulting from the action of barley malt consisted of a comparatively larger amount of maltose, mixed with dextrins of higher rotation and lower reducing power.

The question of the presence of two components in *cholam* malt diastase has also been examined by Patwardhan and Norris [1928], Narayanamurti and Norris [1928] and by Narayanamurti [1930]. Narayanamurti and Norris [1928] found that electro-dialysis greatly increased sachharogenic activity, and that on electro-osmosis, the enzyme was separated into two parts, one of which was predominantly saccharifying while the other was essentially liquefying. Narayanamurti [1930] adopted Bechhold's technique of electro-ultra-filtration and found that the curves obtained for the hydrolysis of starch by diastase before and after electro-ultra-filtration were slightly different, the latter resembling an autocatalytic curve. The components of diastase causing liquefaction and saccharification were partly separated by ultra-filtration, and the liquefying component appeared to be of a higher dispersivity than the saccharifying component.

As many of the studies reported above in regard to *cholam* malt diastase were mainly of a preliminary character, and served to bring out several important differences between *cholam* and barley malts, it was thought that a more detailed and systematic examination of the action of the two malts on starch might serve to throw light on the factors which bring about such great differences in the properties of the two malts. Certain preliminary experiments showed the formation of a good

deal of glucose by the action of *cholam* malt extract on starch. As the question of glucose formation by the enzymic hydrolysis of starch has been a matter of controversy from the time of Brown [Ling and Baker, 1895] and has not so far been investigated with reference to *cholam* malt, this aspect of the problem, including a detailed examination of the maltase activity of *cholam* malt, was taken up first for study (Part I). After this, work relating to a comparison of the amylases from *cholam* and barley malts was taken up for a fresh examination (Part II), and experimental evidence is offered which throws new light on the two-component theory of amylase (Part III).

I. Investigations on the maltase activity of *cholam* malt.

Review of literature.

In the case of barley malt diastase there has been a hot discussion for several years past as to whether by the action of the diastase on starch, glucose is obtained among the products of hydrolysis. Ling and Baker [1896] observed that glucose was formed by the action of diastase from kilned malt on potato starch paste, but later [1897] they tried to explain away the formation of glucose on other grounds and felt themselves satisfied that "glucose cannot be produced by the action of diastase from normal malt on starch". Sullivan [1876] and Brown and Heron [1879] also denied the formation of glucose during diastatic action. Krober [1896], however, maintained that a glucase, an enzyme capable of producing glucose, existed in normal malt. Effront [1899] has also remarked :—"Le infusion de malt agit tres pau sur le maltose, mais le malt concasse agit energiquement sur les sirops de maltose, qui se trans ferment in sirop de dextrose." He has shown the presence of "glucase" (maltase) in several cereals.

That glucose is formed by the action of malt or malt extract or even precipitated malt diastase on starch has since been confirmed by several workers, but it has been explained in two different ways. One school of workers hold that glucose is produced by the action of maltase (which exists in malt or malt extract or even sometimes in precipitated diastase) on maltose produced from starch by the action of diastase of malt. The presence of maltase in plant tissues and most of the cereal grains, malted and unmalted, has been confirmed by several workers [Davis, 1916; Daish, 1916; Ling and Davis, 1923; Leibowitz, 1925; 1926, etc.] Recently, Pringsheim and co-workers [1925, 1927] have been trying to effect a separation of maltase from amylase present in malts, by the use of adsorption and elution methods and also by the use of 87 per cent. glycerine, which they find to yield a

maltase- free extract. With these methods, they were able to obtain a maltase-free amylase but not an amylase-free maltase.

A second school of thought believes that glucose is formed from starch directly by the action of amylase itself, under conditions in which maltase does not act. Brown and Miller [1899] showed that their "Stable Dextrin" on hydrolysis by malt diastase at 55° C. yielded glucose. Ling and Davis [1902] found that when an aqueous solution of precipitated diastase is heated at 65° C. or at a few degrees above this temperature, and the solution allowed to act on starch paste, glucose is invariably present among the final products of hydrolysis. Ling [1903] noted that malto-dextrin-α of Ling and Baker [1895] also yielded about 10 per cent. of glucose when hydrolysed in the presence of malt diastase at 55° C. Davis and Ling [1904] further confirmed the formation of glucose and found that a maximum amount of 12 per cent. glucose could be obtained by restricting malt diastase between 68°-70° C.

Ling and Nanji [1923] are strong exponents of this theory of direct formation of glucose from starch by the action of amylase, without the intervention of maltase. They cite experiments to show that glucose is formed by the action of amylase on starch at temperatures above 60° C., at which temperature maltase is destroyed. They found a maximum production of glucose up to 10-11 per cent. of starch taken, and attribute it to the hydrolysis of β-glucosido-maltose, formed from αβ-hexa-amylose at higher temperatures (60° C.) by the action of diastase.

The question of the *modus operandi* underlying the formation of glucose among starch decomposition products has so far been studied mainly with reference to the amylase of barley malt, and it was considered probable that more decisive light might be thrown on the matter, if the conclusions of previous workers could be verified by reference to other cereal malts besides barley.

Some preliminary experiments showed that a large amount of glucose could be isolated from the products of auto-digestion of *cholam* malt with water, showing the presence of an active maltase in the malt. Hence, a detailed study of the maltase activity of *cholam* malt, as compared with other malts like maize and barley, was first made, before going into the question as to whether glucose could be produced by the action of amylase alone without the intervention of maltase. The investigations relating to maltase activity are contained in the present paper, while the second aspect is examined in the two succeeding papers on *cholam* amylase (Parts II and III).

Examination of some methods for the determination of maltase activity.

The maltase activity of a substance is generally determined by reacting a known weight of it with excess of maltose, and estimating the amount of glucose formed;

but in actual practice the determination is beset with serious difficulties, firstly, on account of the absence of any satisfactory method for the estimation of glucose in presence of other carbohydrates like maltose, dextrin, etc., and secondly, on account of the fact that maltase is an endo-cellular enzyme and hence the raw product containing maltase (malt powder) has to be directly used as source of the enzyme, necessitating the unsatisfactory feature of running controls. Of the various methods employed for the purpose, *e.g.* (1) the glucosazone method; (2) the cupric reduction method; (3) polarimetric method; (4) use of Barfoed's reagent; (5) enzymatic methods; etc., the first two appeared capable of giving quicker and more accurate results than the others, and hence a detailed examination of these was made in order to see whether they could be standardised to reach the desired limit of accuracy.

THE GLUCOSAZONE METHOD.

This depends on the fact that glucosazone is almost insoluble in boiling water while maltosazone is readily soluble and hence is not precipitated. By filtering off and determining the weight of glucosazone formed, one can form an idea of the degree of hydrolysis of maltose; but an exact determination is rendered difficult by several factors which prevent a quantitative precipitation of glucose as osazone, *e.g.*, time of boiling, concentration of reagents added, presence of other carbohydrates, etc. [Lintner and Krober, 1895; Davis and Ling, 1904; Ling and Nanji, 1923]. An attempt was, however, made to standardise the conditions of the estimation so as to obtain reproducible results, and the details of the method, as finally evolved, are given below.

Application of the osazone method for the determination of maltase activity.

In the determination of maltase activity, it was found convenient to take 2·0 grms. of maltose mono-hydrate, to which was added the source of the enzyme (0·2 grm. malt) and the volume was made up to 100 c.c. 10 c.c. aliquots were pipetted out at intervals for the estimation of the glucose contained therein. Now 10 c.c. of the solution, to start with, contained 0·20 grm. maltose hydrate, and when this is hydrolysed to glucose according to the equation

$$C_{12}H_{22}O_{11}.\ H_2O \longrightarrow 2\,C_6H_{12}O_6,$$

the fraction of maltose hydrate undergoing hydrolysis produces the same quantity of glucose, and hence, the sum total of maltose hydrate and glucose remains constant at 0·20 grm. Thus, if we have a table or figure showing the amount of glucosazone obtained for varying proportions of maltose hydrate and glucose, of total weight 0·20 grm., under certain strictly defined "standard conditions" of experimental

procedure, it is possible to estimate the percentage hydrolysis of maltose by this method with a fair degree of accuracy.

Experiment 1. Weights of glucosazone for mixtures of maltose and glucose of total weight 0·20 grm.

Standard conditions.—The following conditions were strictly followed :—Ten c.c. of the solution containing varying mixtures of maltose mono-hydrate and glucose (of known proportions) were pipetted into a boiling tube. Five c.c. of water, one c.c. of phenyl-hydrazine and 2 c.c. of a solution prepared by adding 100 grms. of sodium acetate to 150 c.c. of 50 per cent. acetic acid, were added and the tube was kept in a briskly boiling water bath for 1½ hours, at the end of which time the contents were filtered quickly through a Gooch under good suction, and the precipitate of glucosazone washed with about 20 to 30 c.c. of boiling water. The Gooch was then dried at 110° C. for an hour and weighed.

The figures obtained are contained in Table IV.

TABLE I.

Amount of maltose	Amount of glucose	Weight of glucosazone			Percentage hydrolysis of maltose
		I	II	Average	
Grm.	Grm.	Grm.	Grm.	Grm.	
0·20	*nil.*		*nil*		0
0·19	0·01		No precipitation		5
0·18	0·02		Just a trace.		10
0·17	0·03	0·0140	0·0198	0·0169	15
0·16	0·04	0·0300	0·0260	0·0280	20
0·15	0·05	0·0454	0·0450	0·0452	25
0·14	0·06	0·0548	0·0590	0·0572	30
0·12	0·08	0·0808	0·0810	0·0809	40
0·10	0·10	0·1118	0·1120	0·1119	50
0·08	0·12	0·1322	0·1338	0·1330	60
0·06	0·14	0·1612	0·1616	0·1614	70
0·04	0·16	0·1846	0·1874	0·1860	80
0·02	0·18	0·2106	0·2174	0·2140	90
nil.	0·20	0·2506	0·2490	0·2498	100

By following the procedure outlined above and from the figures given in the table, it is possible to read off the amount of glucose formed and the percentage hydrolysis of maltose. The method, however, is of no use for percentages of hydrolysis below 10 as no glucosazone is formed under these conditions.

THE CUPRIC REDUCTION METHOD.

Since the reducing power of maltose is about 62 per cent. that of glucose, the hydrolysis of maltose into glucose is accompanied by an increase in the cupric reduction value; and methods are available for determining the reducing power of sugars which are of greater accuracy and sensitiveness than the methods applicable in the case of the osazone determination or the Polarimetric Method.

As however, the rate of increase of copper reduction, *viz.*, the increase in weight of cuprous oxide precipitated for, say, a 10 per cent. hydrolysis of maltose into glucose, varies though slightly for different initial concentrations of maltose, it was decided to keep the initial concentration of maltose hydrate constant at 2·0 grms. made up to 100 c.c. Five c.c. of this solution will contain 0·1 grm. of maltose, a quantity convenient for estimation. As maltose mono-hydrate yields on hydrolysis, the same weight of glucose, the sum total of maltose *plus* glucose per 5 c.c. of the solution remains constant at 0·10 grm. during the progressive hydrolysis of maltose.

Experiment 2. Reducing powers of maltose and glucose mixtures of total weight 0·10 grm.

Mixtures of known amounts of maltose hydrate and glucose were taken for analysis, and the copper reduction values were determined by the 'permanganate' method. The figures obtained are given in Table II.

TABLE II.

Amount of maltose mgrm.	Amount of glucose mgrm.	Reducing power in mgrm. Cu.	Increase of reducing power in mgrm. Cu.	Percentage hydrolysis of maltose
100	*Nil*	108	*Nil*	0
90	10	119	11·0	10
80	20	129	21·0	20
70	30	138·5	30·5	30
60	40	148	40·0	40
50	50	157·5	49·5	50
40	60	166·5	58·5	60
30	70	175·5	67·5	70
20	80	184·5	76·5	80
10	90	192·5	84·5	90
5	95	197·0	89·0	95
0	100	200·0	92·0	100

The conditions of the "cupric reduction" method, as applied to the determination of maltase activity, were, therefore, as follows:—

"A weighed amount of the enzyme (0·2 grm. of malt) is added to 2·0 grms. of maltose mono-hydrate, and the volume made up to 100 c.c., a few drops of toluene being added as preservative. Aliquots of 5 c.c. of the clear solution are pipetted out at intervals of 24 hours or longer, and the reducing power determined by the volumetric "permanganate" method, (*loc. cit.*). "A control is run with the source of the enzyme only, and the value of the control is deducted from that of the experiment. From the net increase in reducing power, by reference to Table II the amount of glucose formed and hence the percentage hydrolysis of maltose is read out.

In the following experiments on maltase activity, the cupric reduction method has been mainly used, with frequent checks by other methods, *e.g.*, the glucosazone or polarimetric.

Experiments on maltase activity.

Experiment 3. Presence of maltase in malted and unmalted cholam.

Cholam malt was prepared according to the method of Viswanath, Rao and Aiyangar [1918]. 0·5 grm. of malted or unmalted *cholam* powder was added to 2·5 grm. of maltose, the volume made up to 100 c.c. and 5 c.c. portions pipetted out at intervals for cupric reduction and glucosazone determinations.

Table III.

Hours from start	Malted *cholam* (Cupric reduction of 5 c.c. in mgrm. Cu)				Unmalted *cholam* (Cupric reduction of 5 c.c. in mgrm. Cu)			
	Control	Expt.	Diff.	Increase	Control	Expt.	Diff.	Increase
Start	3	134	131	..	3	131	128	..
48 hours . . .	3	144	141	10	4	138	134	6
96 „ . . .	4	151	147	16	3	148	145	17
144 „ . . .	4	165	161	30	3	163	160	32
192 „ . . .	4	173	169	39	4	175	171	43
240 „ . . .	5	181	176	45	4	179	175	47
	Wt. of glucosazone per 5 c.c. after 240 hours . 0·0822 grm.				Wt. of glucosazone per 5 c.c. after 240 hours . 0·0826 grm.			

The results show the presence of an active maltase in both malted and unmalted *cholam*, which are almost of equal activity.

Experiment 4. *Absence of maltase in the water extract of unmalted* cholam, *but its presence in the extract of malted* cholam.

Twenty grm. of flour from raw *cholam* and *cholam* malt were extracted with 200 c.c. of water at 30° C. for about 20 hours and then filtered. Fifty c.c. of the filtrate were added to 2·5 grms. of maltose and the volume made up to 100 c.c. Toluene was added as preservative. Controls were run. Digestion proceeded at 30° C. and aliquots of 10 c.c. were taken for cupric reducing power.

TABLE IV.

Hours from start	Unmalted *cholam* (Cupric reduction values				Malted *cholam* of 5 c.c. in mgrm. Cu)			
	Control	Expt.	Diff.	Increase	Control	Expt.	Diff.	Increase
Start	25	148	123	..	40	164	124	..
48 hours . . .	10	133	123	*Nil*	35	161	126	2
96 ,, . . .	10	131	121	..	37	169	132	8
144 ,, . . .	11	131	120	..	37	173	136	12
192 ,, . . .	11	135	124	1	38	177	139	15
240 ,, . . .	13	138	125	2	37	181	144	20
	Wt. of glucosazone per 20 c.c. after 240 hours . . .			*Nil*	Wt. of glucosazone per 20 c.c. after 240 hours .			0·1444 grm.

The above figures show that the extract from malted *cholam* is rich in maltase, while that from unmalted *cholam* does not contain maltase. As experiment (3) has shown the presence of an almost equally active maltase in both malted and unmalted *cholam*, it would seem that the process of germination helps to render the maltase extractable with water.

Experiment 5. *Absence of maltase in alcohol-precipitated diastases from* cholam *and barley malts.*

The diastases were prepared from *cholam* and barley malts according to the method of Lintner [1886]. 0·02 grm. of each diastase was added to 2·0 grm. of

maltose and the volume made up to 100 c.c. The other details were the same as in the previous experiments. The results which are contained in Table V show the absence of maltase in alcohol-precipitated diastase.

TABLE V.

Hours from start	*Cholam* malt diastase (Cupric reduction of 5 c.c. solution in mgrm. Cu)				Barley malt diastase (Cupric reduction of 5 c.c. solution in mgrm. Cu)			
	Control	Expt.	Diff.	Increase	Control	Expt.	Diff.	Increase
Start	2	102	100	..	3	103	100	..
48 hours	2	102	100	*Nil*	3	102	99	..
96 ,,	2	103	101	1	3	102	99	..
144 ,,	2	103	101	1	2	103	101	1
192 ,,	2	104	102	2	2	102	100	..
240 ,,	2	104	102	2	2	103	101	1
Wt. of glucosazone per 20 c.c. after 240 hours	*Nil*				Wt. of glucosazone per 20 c.c. after 240 hours	*Nil*		

Experiment 6. A comparison of the maltase activities of cholam, *maize and barley malts.*

0·5 grm. of each malt was added to 2·0 grm. of maltose and digestion proceeded at 30° C. Other details were as usual.

TABLE VI.

Comparison of cholam, *barley and maize maltases.*

Hours from start	Increase in cupric reduction of 5 c.c. solution in mgrm. Cu Initial value 102 mgrm. Cu		
	Cholam	Barley	Maize
After 48 hours	13 mgrms.	3 mgrms.	16 mgrms.
,, 96 ,,	25 ,,	7 ,,	23 ,,
,, 144 ,,	43 ,,	12 ,,	43 ,,
,, 192 ,,	49 ,,	17 ,,	55 ,,
,, 240 ,,	58 ,,	22 ,,	63 ,,
Wt. of glucosazone per 5 c.c. after 240 hours	·0810 grm.	·0328 grm.	·0850 grm.

The figures show that the maltases in maize and *cholam* are of the same order of activity, while that in barley is much weaker. Thus, in 10 days, *cholam* and maize maltases hydrolyse about 60 per cent. of the maltose, while barley carries on the hydrolysis only to the extent of 20 to 25 per cent.

Experiment 7. Comparison of the maltase activity of cholam, *maize and barley malts at different pH ranges.*

0·5 grm. of each malt was added to 2·0 grm. of maltose and 20 c.c. of McIlvaine's buffer solution corresponding to the pH required, and the volume was made up to 100 c.c. Other details were as usual. The increase in cupric reduction of 10 c.c. of the solution in the different cases is represented graphically in Fig. 1.

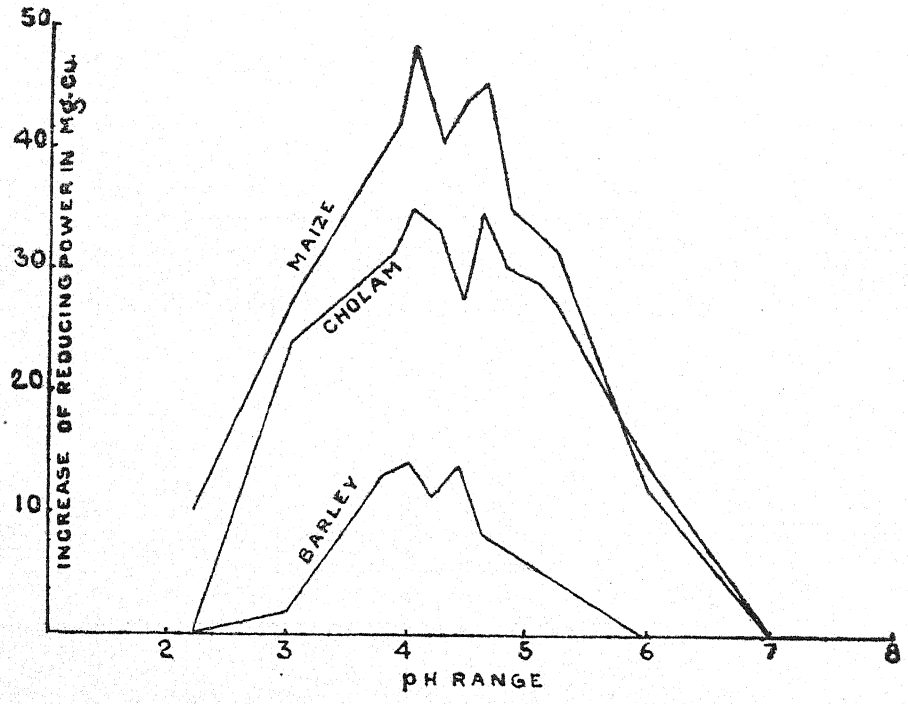


Fig. 1. pH and maltase activity.

The curves show that :—(1) The maltases in *cholam*, maize and barley malts are similar in their range of activity ; all of them are active in the range of pH 3·0 to 5·0 ; outside this range, the activity considerably diminishes. The rate of inactivation is specially rapid on the alkaline side ; even pH 7·0 is found to considerably diminish maltase activity.

(2) The pH-activity curves appear in all the three cases to be double-topped ones, showing two maxima ; it is proposed to examine the reason for this peculiar behaviour later on.

(3) In all the three cases, the optimum zone of activity lies at pH 4·0-4·4.

Experiment 8. Optimum temperature for the maltase activity of cholam *and maize malts.*

0·5 grm. of *cholam* or maize malt was added to 1·0 grm. of maltose and 20 c.c. of pH 4·0 buffer, and made up to 100 c.c. Toluene was added as preservative, and the flasks were incubated for a period of 24 hours at the temperatures noted in the following table, after which 10 c.c. were taken for determination of reducing power. The necessary controls were run. The results obtained are shown in Fig. 2.

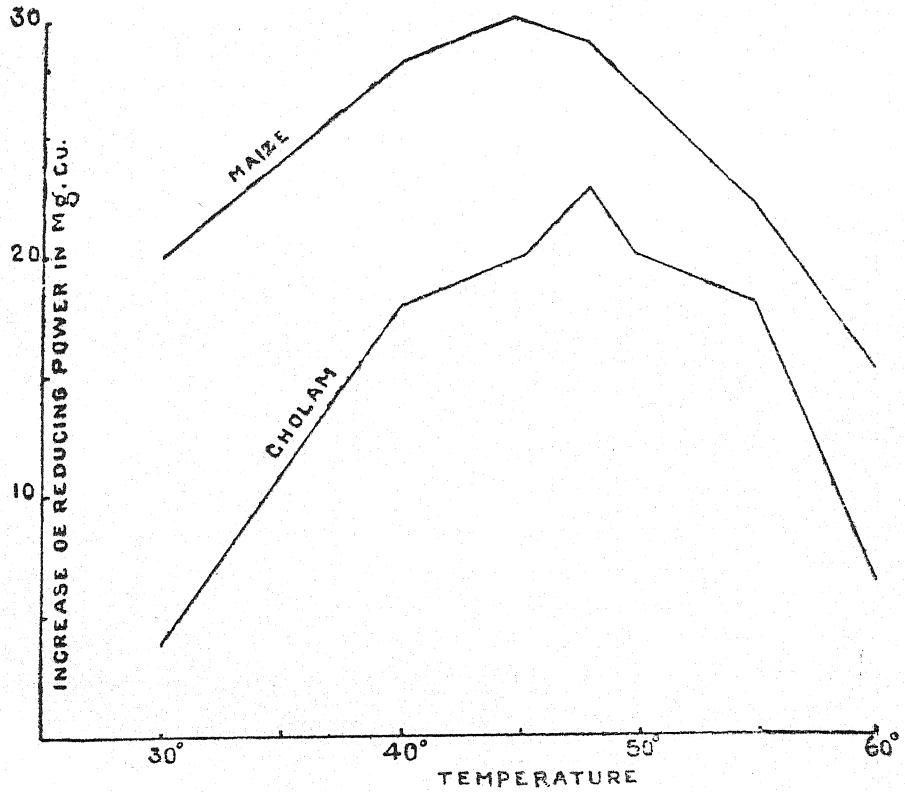


Fig. 2. Temperature and maltase activity.

It is evident from the curves that the maltases of *cholam* and maize are most active between 40° and 50° C., the optimum temperature for *cholam* maltase lies

between 45° C. and 50° C. while that for maize maltase lies at about 45° C. The figure also shows that under conditions of optimum pH and temperature, the maltase of maize exhibits a higher activity than that in *cholam*.

Experiment 9. Influence of concentration of enzyme on maltase activity in cholam *malt.*

Weighed quantities of *cholam* malt, as shown in Table VII below, were added to 1·0 grm. of maltose and 20 c.c. of pH 4·0 buffer, the volume made up to 100 c.c. and the contents incubated at 45° C. for 24 hours, after which 10 c.c. were taken for analysis. Necessary controls were run.

TABLE VII.

	(Cupric reduction of 10 c.c. of solution in mgrm. Cu)								
Malt taken grm.	Cont.	Expt.	Diff.	Increase	Malt taken grm.	Cont.	Expt.	Diff.	Increase
Nil	..	106	106	..	*Nil*	..	106	106	..
0·25	8	131	126	16	0·1	3	119	116	10
0·50	15	144	129	23	0·2	5	125	120	14
0·75	22	153	131	25	0·3	9	133	124	18
1·00	33	173	140	34	0·4	13	144	127	21
					0·6	17	148	131	25
					0·8	26	160	134	28
					1·0	35	172	137	31

The results show that there is no linear relationship between the amount of malt taken and the percentage of maltose hydrolysed, even when small quantities of malt are taken. Smaller quantities of malt produce relatively greater percentages of hydrolysis : the curve shows a steeper slope in the beginning and then gradually flattens out.

Experiment 10. Influence of concentration of maltose (substrate) on maltase activity of cholam *malt.*

0·25 grm. of malt was mixed with various quantities of maltose from 0·5 grm. to 2·0 grm., the volume made up to 100 c.c. and the contents incubated at 45° C. for 24 hours. Controls were run.

TABLE VIII.

Maltose taken grm.	Volume of solution containing 100 mgrm. maltose c.c.	Reducing power of this volume of solution in mgrm. Cu	Control mgrm. Cu	Diff. mgrm. Cu	Total glucose formed mgrm.
0·5	20	148	16	132	130
1·0	10	131	8	123	170
1·5	6·7	128	5	123	250
2·0	5·0	122	4	118	240

The figures show that the glucose produced by 0·25 grm. malt acting on maltose, increases up to 1·5 grm. maltose, after which there is no further increase. Hence, a ratio of 1 : 6 between enzyme and substrate is preferable, while using *cholam* malt for determinations of maltase activity.

Experiment 11. Influence of added substances on maltase activity of cholam.

The effect of substances noted in the accompanying table was next examined by adding them in the quantities given therein to 1·0 grm. maltose *plus* 0·25 grm. malt, and making up to 100 c.c. Ten c.c. portions were taken for analysis.

TABLE IX.

Substance added	Amount	Period of incubation	Difference from control value
(A) *Preservatives*—			
Toluene	A few drops	96 hrs. at 30°C.	Increase of 51 mgrm. Cu over initial value.
Formaldehyde	,,	,,	Increase of 50·5 mgrm. Cu over initial value.
Chloroform	,,	,,	Increase of 51 mgrm. Cu over initial value.
(B) *Alcohols*—			
Ethyl alcohol	10 c.c.	24 hrs. at 45°C: (control shows 20 mg. increase)	—5 (difference from control value).
Glycol	,,	,,	—10 ,,
Glycerol	,,	,,	—14 ,,
Mannitol	,,	,,	*Nil.*
(C) *Amino acids*—			
Glycine	0·5 grm.	,,	+8 ,,
Asparagine	,,	,,	+1 ,,
Aspartic acid	,,	,,	—10 ,,
(D) *Other enzymes*—			
Diastase	0·10 grm.	,,	+5 ,,
Pepsin	,,	,,	+12 ,,
Trypsin	,,	,,	+ 2 ,,
Emulsin	,,	,,	*Nil.*

The figures show that preservatives like toluene, formaldehyde and chloroform are equally good for their purpose in relation to maltase activity. though toluene was found to be the most convenient in actual use. Alcohols like ethyl alcohol, glycol and glycerol exert a deleterious influence on maltase activity, whose extent increases with the number of hydroxyl groups in the molecule. This is in accord with the observation of Pringsheim [1925] that 87 per cent. glycerol effectively destroyed maltase in a mixture of maltase and amylase. It is noteworthy that mannitol, in spite of the longer number of hydroxyl groups in the molecule has no harmful effect on maltase activity. Among the amino-acids examined, glycine at a concentration of 0·5 per cent. exerts a favourable influence on maltase activity, while asparagine has no influence, and aspartic acid has actually a depressing influence, probably due to its acidity.

Among the enzymes, pepsin has a marked accelerating effect, which may be due to the amino-acids produced by the enzymic hydrolysis of proteins in malt, while diastase has only a small accelerating effect; but trypsin and emulsin have practically no action. The inactivity of emulsin towards maltase is in contrast to its strong deleterious action towards diastase.

Experiment 12. Thermo-stability of maltase.

(*A*) *In the dry state.*—0·25 grm. of dry *cholam* malt was kept at the different temperatures noted below for 15 or 30 minutes, and then added to 1·0 grm. of maltose, buffered to pH 4·0 and made up to 100 c.c. Digestion proceeded at 30°C. and 10 c.c. were taken for analysis. Controls were run.

TABLE X.

Temperature (°C.)	Period of heating (minutes)	Cupric initial value	Reduction of 10 c.c. in mgrm. of Cu	
			Increase after 48 hours	Increase after 120 hours
50	15	110	20	66
60	15	110	25	64
70	15	110	25	64
80	15	110	23	61
90	15	110	22	60
100	15	110	20	55
110	15	110	13	44
120	15	110	6	30
120	30	110	5	16
130	30	110	1	4
140	30	110	*Nil*	*Nil*

The data show that heating for 15 minutes in the dry state below 70°C. has no appreciable effect on maltase activity: above that temperature, the maltase is gradually destroyed. The rate of destruction is slow, probably due to the endo-cellular nature of the enzyme; thus, heating in the dry state at 100°C. for 15 minutes reduces the activity only by about 10 per cent. For complete destruction of the enzyme, the dry malt has to be exposed to 140°C. for 30 minutes.

(*B*) *Malt contact with water.*—0·25 grm. of *cholam* malt was mixed with 20 c.c. of pH 4·0 buffer solution and kept in a water bath at the temperatures noted below for 15 minutes. Then it was cooled, 1·0 grm. of maltose added, the volume made up to 100 c.c. Other details were as in the previous experiment.

TABLE XI.

Temperature (°C.)	Cupric reduction of 10 c.c. solution in mgrm. Cu				
	Start	After 48 hrs.	Increase	After 96 hrs.	Increase
30	108	128	20	151	43
60	108	126	18	149	41
65	108	115	7	138	30
70	108	109	1	116	8
75	108	108	*Nil*	108	*Nil*

The figures show that if the malt be in contact with water, the maltase, though endo-cellular, is destroyed at a much lower temperature (15 minutes at 75°C.) than if the malt be dry (30 min. at 140°C.). This difference may probably be due either to the ability of the water to quickly penetrate the cell walls and raise the contents of the cell rapidly to the outside temperature or to the rapid decomposition of the protein complex in the cells by the action of water at the higher temperature.

(*C*) *Maltase in aqueous solution.*—20 grms. of *cholam* malt were extracted with 200 c.c. of water for 20 hours, in presence of toluene, then filtered, and 50 c.c. portions of the filtrate were taken in 100 c.c. flasks and kept in water-baths at the temperatures noted below for 15 minutes. At the end of the period, the solution was cooled, 2·5 grms. of maltose and toluene added, and the volume made up to 100 c.c. Digestion proceeded at 30°C. and 5 c.c. portions taken for analysis.

TABLE XII.

Hours from start	Temperature 55°C. (15 min.)			Temperature 60°C. (15 min.)		
	Cupric reduction of 5 c.c. of solution in mgrm. Cu					
	Control	Expt.	Difference	Control	Expt.	Difference
Start . .	53	177	124	53	180	127
48 . . .	45	179	134	52	176	124
96 . . .	44	184	140	53	180	127
144 . . .	45	186	141	53	179	126
192 . . .	46	188	142	57	183	126
240 . . .	47	188	141	59	184	125
288 . . .	48	190	142	60	185	125
Glucosazone from 20 c.c. .	0·0712	0·1346	0·0634 grm.	0·0852	0·0860	0·0008 grm.

The figures show that keeping the malt extract at 55°C. for 15 minutes, though it greatly inhibits maltase activity, does not destroy it completely. Keeping at a temperature of 60°C. for 15 minutes destroys the maltase in the amounts present in the malt extract. It has to be noted in this connection that only a small quantity of maltase goes into solution in water, even after 20 hours standing, and hence the above brief exposure at 60°C. might be sufficient to destroy this small amount of maltase.

SUMMARY.

1. The methods for the determination of maltase activity have been studied and two methods, *viz.*, the glucosazone and cupric reduction methods, have been standardised to give satisfactory measurements of the activity.

2. It has been shown that an active maltase is present in both unmalted and malted *cholam*, whose strength compares favourably with that in maize and is much superior to that in barley.

3. The maltase in malted *cholam* is slightly soluble in water, but that in unmalted *cholam* is not.

4. The effects of pH, temperature, concentration of enzyme and concentration of substrate have been examined. The maltase in *cholam* reacts best at pH 4·2 and at temperature 48°C. The amount of maltose hydrolysed does not bear a linear relationship to the malt used, even for small quantities of malt, but tends to curve

down from the beginning. A proportion of 1 : 6 between enzyme (malt) and substrate (maltose) is recommended to ensure maximum activity.

5. The maltase in *cholam* malt has been compared with that in maize and barley, in regard to activity at different pH ranges and different temperatures. It was found that all the three maltases showed maximum activity at about pH 4·0-4·4 and at temperatures from 45° to 50°C.

6. The influence on maltase activity of *cholam* of the addition of other substances like preservatives, *e.g.*, toluene, chloroform and formaldehyde ; of alcohols, *e.g.*, ethyl alcohol, glycol, glycerol and mannitol ; of amino-acids, *e.g.*, glycine, asparagine and aspartic acid ; and of enzymes *e.g.*, pepsin, trypsin, emulsin and diastase has been examined. It was found that any of the three preservatives mentioned above, preferably toluene, could be employed ; that the alcohols (except mannitol) had a depressing effect on maltase, which increased with the number of hydroxyl groups present in the alcohol, glycerol being very rapid in the inactivation of maltase. Among the amino-acids, glycine accelerates the velocity of action, while asparagine has no effect and aspartic acid has a depressing influence. Pepsin exerts a marked and diastase a slight accelerating effect, while emulsin has no action.

7. The effect of higher temperatures on the rapidity of destruction of maltase in *cholam* malt was studied. It was found that 30 minutes heating in the dry state at 140°C. destroys the maltase completely, whereas if the malt be mixed with water, 15 minutes heating at 75°C. is sufficient to effect complete inactivation. Partial destruction of the enzyme begins at 60°C. In the water extract of *cholam* malt, there is only a small quantity of maltase dissolved, and heating the filtered extract at 60°C. for 15 minutes, destroys the enzyme.

II. A comparison of the amylases in barley and *cholam* malts.

That diastase exerts a two-fold action on starch—namely a liquefying and a saccharifying action—has been recognised for a long time past. Brown and Morris [1890] distinguished between "Translocation Diastase" possessing no liquefying power but acting on gelatinised starch, and "Secretion Diastase", which possessed such liquefying power.

Succeeding work on a comparison of amylases from different sources showed that the liquefying and saccharifying powers varied widely from one amylase to another and that they varied independently of one another and even in opposite directions. Thus, it has been noted [Viswanath, Rao and Aiyangar, 1918 ; Norris and Viswanath, 1923] that *cholam* malt diastase exerts a much greater liquefying action, but less saccharifying effect than barley malt diastase. Patwardhan [1928—1930] has compared the relative differences in the liquefying and saccharifying powers of amylases from wheat, maize, rice and other cereal malts.

This capacity for independent variation of the two powers (liquefaction and saccharification) has been explained on the hypothesis that ordinary diastase contains two different components—one a liquefying enzyme and the other a saccharifying one—and attempts have been made to study the components independently and to separate them from one another.

Narayanamurti and Norris [1928] tried to separate the two components of *cholam* malt amylase by using methods of electrodialysis and electro-osmosis. Narayanamurti [1930] adopted Becchold's technique of electro-ultra-filtration for the same purpose. These workers found that the activity of *cholam* amylase could be considerably increased by the above methods, and that it could be partially separated into its two components—the one a powerful liquefier and the other a good saccharifier. Their results have been confirmed by Luers and Lechner [1933] working with malt extracts.

Viswanath and Suryanarayana [1925] studied the mechanism of the action of *cholam* amylase on starch and arrived at the conclusion that in the hydrolysis of starch by *cholam* malt extract, two enzymes are concerned—an amylase converting starch into dextrin and a dextrinase which converts the dextrin into maltose. The authors have also indicated that the production of maltose appeared to be the result of a series of reactions passing from one phase to another in regular sequence.

Ohlsson, in his experiments on barley malt diastase [1922, 1926, 1930] claims to have separated the two components by the differential effects of pH and temperature, into a dextrinogenase which carries the hydrolysis only to the dextrin stage and a saccharogenase which hydrolyses the starch or dextrin to maltose. By changing the pH of malt extract to 3·3 and keeping at 0°C., a preparation of saccharogen-amylase was obtained, containing very little active dextrinogenase. By heating malt extract at a pH of 6 to 7 at 70°C., a preparation of dextrinogen-amylase was obtained containing very little saccharogenase.

Ohlsson's work has been confirmed by Edfelt, Nordh and Swaetichin [1930]. Sabalitschkka and Weidlich [1929], however, hold that the dextrinising and saccharifying enzymes are one and the same. They found that the ratio of saccharification to dextrination constants in 15 experiments with amylase and diastase preparations, which received different pre-treatments, was $1{\cdot}28 \pm 0{\cdot}17$ and was not changed when the enzyme was concentrated 15 to 17 times by adsorption and elution, 6·6 times by dialysis and 4·5 times by precipitation with alcohol. They say that the constancy of this ratio supports the view that one and the same enzyme is responsible for both dextrination and saccharification.

In this connection, it is interesting to note also the views of Ling and Nanji [1923, 1925], who carried out a series of investigations on the nature of products

obtained by enzymatic hydrolysis of starch. These authors arrive at the conclusion that starch consists mainly of two components—polymerised amylose and amylopectin—of which amylose yields by diastatic hydrolysis only maltose, while amylopectin yields a series of degradation products including the various dextrins, iso-maltose, β-glucosidomaltose and maltose. They found that glucose was produced under certain conditions of hydrolysis.

It was considered that a detailed comparison of the amylases from two different sources like *cholam* and barley, in regard to the kinetics of their action on starch and the nature of the products formed at different stages of the action, might throw more light on this question of the presence of two enzymes or only one in amylase; and might also incidentally offer further experimental evidence relative to the nature of starch.

THE LIQUEFACTION OF STARCH BY *CHOLAM* AND BARLEY DIASTASES.

Malts were prepared from *cholam* and barley according to the procedure recommended by Viswanath, Rao and Aiyangar [1918]. Amylase preparations were made from the malts thus prepared, according to the method of Lintner [1886], by precipitation with alcohol. The recovery was about one grm. of precipitated diastase from 200 grm. of *cholam* malt and about 2·0 grm. from 200 grm. of barley malt.

Experiment 1. Rate of liquefaction of starch by cholam *and barley malt diastases at different pH ranges.*

0·02 grm. of precipitated *cholam* diastase and 0·04 grm. of barley malt diastase were shaken with 20 c.c. of water in presence of toluene and filtered. One c.c. of the filtered solution was added to 40 c.c. of 3 per cent. soluble starch (Merck's), buffered to the required pH by the addition of 10 c.c. of McIlvaine's Buffer. The time of adding the enzyme was taken as the time of start of the experiment. After mixing well, 10 c.c. of the solution were transferred to an Ostwald's Ostwa Pipet Viscosimeter kept in a thermostat at 35°C. The time of determination of viscosity was taken to be the "mean time" between the beginning and end of fall between marks in the viscosimeter. In all cases, controls were run to determine the initial viscosity of the starch mixture, by adding in place of the enzyme either the boiled enzyme, or an equal quantity of distilled water. The starch liquefying power at the different pH ranges was measured by the percentage fall of viscosity at the end of 30 minutes. The change in iodine colour was also noted.

The results obtained are represented graphically in Fig. 3.

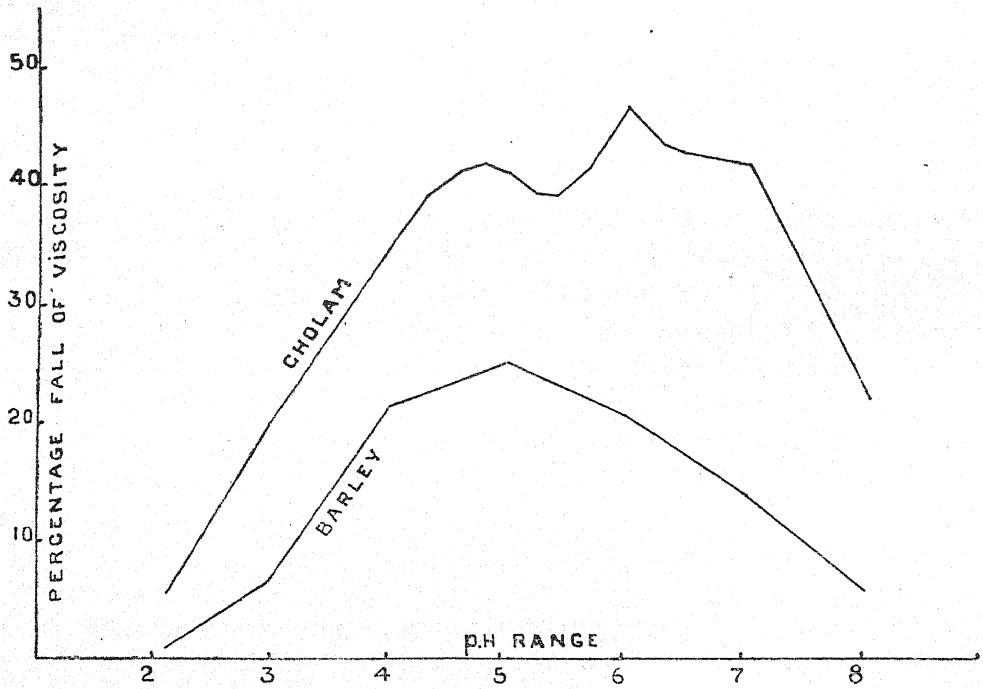


Fig. 3. pH and liquefaction.

The curves show:—(1) At all corresponding pH values, *cholam* malt diastase causes a much greater fall of viscosity than the barley enzyme even though the amount of barley enzyme taken for the present experiment is twice that of *cholam*.

(2) Both the diastases are active in their liquefying power in the range of pH 4·0—6·0; outside this range, the activity rapidly falls off, the fall being more rapid on the acid side.

(3) The curve for *cholam* is a double-topped one showing two maxima, a lower one at pH 6·0, whereas the curve for barley shows one maximum only at pH 5·0. This difference between *cholam* and barley enzymes, which was confirmed by repeated trials, is noteworthy and an explanation is offered in Part III.

Experiment 2. Liquefying action of cholam *and barley malt diastases at different temperatures.*

0·01 grm. of precipiated *cholam* malt diastase and 0·06 grm. of the barley enzyme were separately dissolved in 20 c.c. of water and filtered. One c.c. of the enzyme solution was added to 40 c.c. of 4 per cent. starch solution, buffered to pH 5·0 by the addition of 10 c.c. of buffer, and after mixing well, 10 c.c. were taken

into the viscosimeter and readings taken at different temperatures. Controls were run to determine the initial viscosity of the starch mixture at the various temperatures, by substituting one c.c. of water in place of one c.c. of enzyme. The following values were obtained :—

TABLE I.

Temperature	Value of control	Percentage fall on initial viscosity	
		Cholam	Barley
30°C.	8 min. 10 sec.	28·4	23·3
40°C.	6 „ 50 „	39·2	30·0
45°C.	6 „ 8 „	41·1	34·1
50°C.	5 „ 36 „	42·6	33·8
55°C.	5 „ 10 „	40·3	30·6
60°C.	4 „ 50 „	36·5	26·8
70°C.	4 „ 16 „	26·6	12·3

The figures show that the optimum temperature for liquefaction by *cholam* diastase lies at 50°C., while that for barley diastase lies at about 45°C. (Fig. 4). At higher temperatures (70°C) *cholam* diastase appears to be better able to preserve its liquefying power than barley diastase.

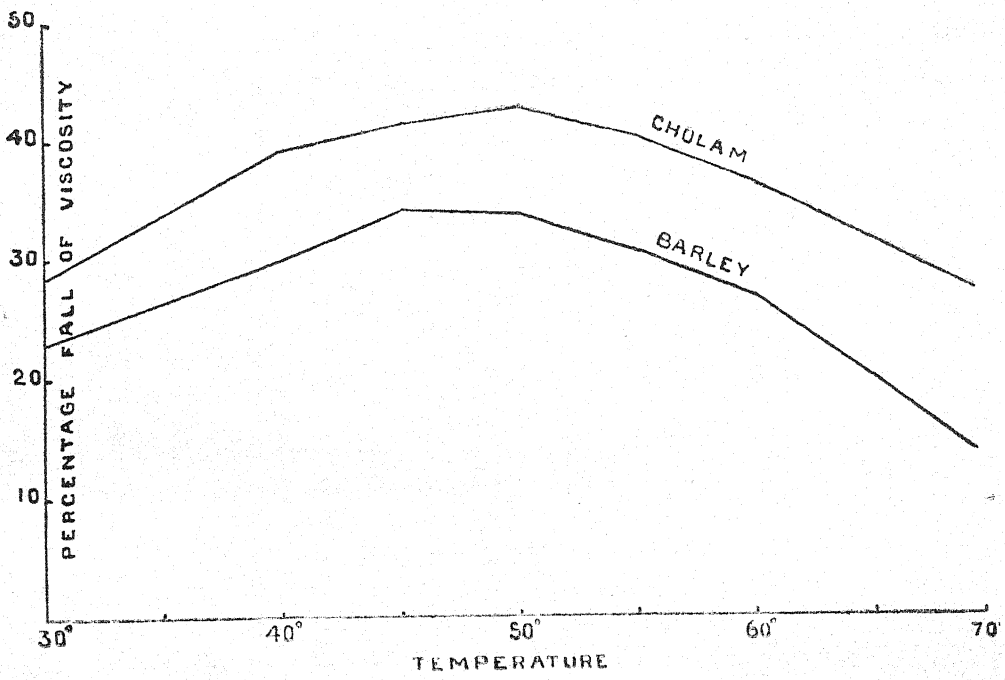


Fig. 4. Temperature and liquefaction.

Experiment 3. Influence of concentration of enzyme on the liquefying action of cholam *and barley malt diastases.*

Six mgrm. of *cholam* diastase and 40 mgrm. of barley diastase were separately dissolved in 20 c.c. of water and filtered. To 40 c.c. of 4 per cent. starch solution, 7·5 c.c. of pH 5·0 buffer and the following quantities of enzyme and water (to make up 2·5 c.c.) were added.

Enzyme	c.c.	*nil*	0·5	1·0	1·5	2·0	2·5
Water	c.c.	2·5	2·0	1·5	1·0	0·5	*nil*

The mixture was stirred well and viscosity readings taken at 35°C. with 10 c.c. The control value was 6 min. 58 sec.

TABLE II.

Quantity of enzyme added (in c. c.)	Percentage fall of viscosity in ½ hr.	
	Cholam	Barley
0·5	17·2	13·9
1·0	29·8	21·1
1·5	34·5	27·3
2·0	37·4	32·1
2·5	40·1	36·3

The curves relating concentration of enzyme and liquefying power are similar in the cases of both *cholam* and barley diastases. They are steep at the earlier stages, but soon tend to flatten out; *i.e.*, small amounts of the enzyme are able to produce relatively larger percentages of hydrolysis, as measured by the fall of viscosity than larger amounts of enzyme. It is noteworthy that there is no linear relationship between fall of viscosity and concentration of enzyme, even at the earliest stages; this is in marked contrast to the linear relationship that has been noticed in the case of saccharifying power, known commonly as Kjeldahl's "Law of proportionality". This absence of a linear relationship even at the earlier stages shows that the liquefying powers of two diastases cannot be directly compared by (as they are not proportional to) the respective percentage falls in viscosity which

they produce acting on the same sample of starch under the same conditions.

Experiment (4). Influence of concentration of substrate on the liquefying power of cholam *and barley malt diastases.*

0·01 grm. of *cholam* diastase and 0·06 grm. of barley diastase were separately dissolved in 20 c.c. of water and filtered. One c.c. of the filtered enzyme was added to 40 c.c. of starch solution of different concentrations (0·5 per cent., 1·0 per cent., 2·0 per cent., 3·0 per cent. and 4·0 per cent.) to which had been added 10 c.c. of pH 5·0 buffer. Ten c.c. were taken for viscosimetric reading at 35°C. Controls were run as usual for the different concentrations of the starch mixtures. The following values were obtained.

TABLE III.

Concentration of starch	Control value	Percentage fall of viscosity in ½ hour	
		Cholam	Barley
(Per cent.)			
0·5	2 minutes 53 seconds	9·2	10·1
1·0	3 „ 14 „	15·3	16·2
2·0	4 „ 3 „	26·1	25·4
3·0	5 „ 6 „	32·4	30·1
4·0	6 „ 40 „	37·3	33·2

The figures show that :—(1) The liquefying power, as indicated by rate of fall of viscosity, increases with increase of substrate, in the case of both *cholam* and barley diastases. (2) The increase is rapid for small concentrations of substrate and tends later to flatten out ; above 3 per cent. concentration, further increase in the rate is slow and hence 3 per cent. concentration of starch can be taken to provide satisfactory scope for liquefying activity. (3). The increase in the rate of fall of viscosity for increasing concentrations of substrate is greater in the case of *cholam* than in the case of barley, probably due to the greater liquefying capacity of the former enzyme.

Experiment (5). Temperature of destruction of the liquefying power of cholam *and barley malt diastases.*

0·02 grm. of the *cholam* enzyme and 0·20 grm. of the barley enzyme were separately dissolved in 40 c.c. of water and filtered. 5 c.c. portions of the filtrate

were transferred to test-tubes and kept at the following temperatures for a period of 15 minutes, after which they were cooled, and one c.c. of the enzyme solution added to 40 c.c. of 3 per cent. starch solution, buffered to pH 5·0 by the addition of 10 c.c. of buffer. The control value was 7 mins. 6 secs.

TABLE IV.

Temperature of heating (15 minutes)	Percentage fall of viscosity in ½ hour	
	Cholam	Barley
35	40·1	30·2
50	46·3	42·4
60	36·1	37·2
65	20·6	26·4
70	6·7	13·6
75	2·8	*Nil*
80	*Nil*	*Nil*

The figures show that heating the enzyme solution above its optimum temperature involves the destruction of a portion of its activity. The rate of destruction is specially rapid above 65°C. In the case of *cholam*, heating at 80°C. for 15 minutes appears to be necessary to destroy the liquefying enzyme, while in the case of barley heating at 75°C. for 15 minutes seems to be enough to achieve the purpose.

Experiment (6). *The velocity of liquefaction of starch by* cholam *and barley malt diastases.*

Five mgrms. of *cholam* diastase and 75 mgrm. of barley diastase were dissolved in 10 c.c. of water and filtered. One c.c. of the clear enzyme solution was added to 40 c.c. of 3 per cent. starch buffered with 10 c.c. of pH 5·0 buffer. Ten c.c. were transferred into the viscosimeter and readings taken at 35°C. The change in iodine colouration was also noted from time to time.

TABLE V.

Cholam malt diastase			Barley malt diastase		
Time in min. and sec.	Viscosity in min. and sec.	Iodine colour	Time in min. and sec.	Viscosity in min. and sec.	Iodine colour
Start	8—2	Blue	Start	8—10	Blue
4—38	5—17	,,	5—42	7—0	,,
16—26	3—49	,,	13—33	6—13	,,
20—59	3—40	,,	20—34	5—41	,,
29—53	3—34	,,	27—22	5—21	,,
43—13	3—23	,,	34—1	5—18	,,
1 hour	3—18	Bluish violet	1 hour	4—30	,,
1½ hours	3—17	Violet	1½ hours	4—15	,,
2 ,,	3—16	Red	2 ,,	4—1	,,
3 ,,	3—15	,,	3 ,,	3—38	,,
4 ,,	3—14	Brown	4 ,,	3—25	,,
5 ,,	3—13	Light brown	5 ,,	3—20	,,
6 ,,	3—12	No colour	6 ,,	3—17	Bluish violet
7 ,,	3—13	..	7 ,,	3—15	Violet
8 ,,	3—14	..	8 ,,	3—15	,,
			9 ,,	3—14	Red
			24 ,,	3—14	Brown

From a comparison of the values for the rate of fall of viscosity in the two cases, one would note that *cholam* is a much more powerful liquefier than barley (Fig. 5). But even though the liquefying power is widely different in the two cases, the respective viscosity curves resemble one another in several important respects, particularly in regard to the relationship between viscosity and iodine colour change in the two cases. With both *cholam* and barley diastases, there is at first a very rapid fall of viscosity ; in fact, nearly the whole of the total fall in viscosity is accomplished while there is still the blue colour with iodine, indicating that the initial change is probably a rapid depolymerisation of starch into simpler molecules which continue to give the blue colour with iodine. The second stage of hydrolysis begins when the blue colour changes to purple, violet and red, showing the hydrolysis of these simpler molecules into dextrin and sugar. At this stage, the viscosity curve shows a sharp bend, and after the colour change to violet, the curve is almost horizontal and shows little further fall. This relationship between the viscosity curve and iodine colour change is shared in common by both *cholam* and barley malt diastases, showing the close agreement between these two methods of determining the rate of hydrolysis of starch. The viscosity method, of course, gives a better measure of the rate at intermediary stages of hydrolysis, than the ' iodine colour-change ' method.

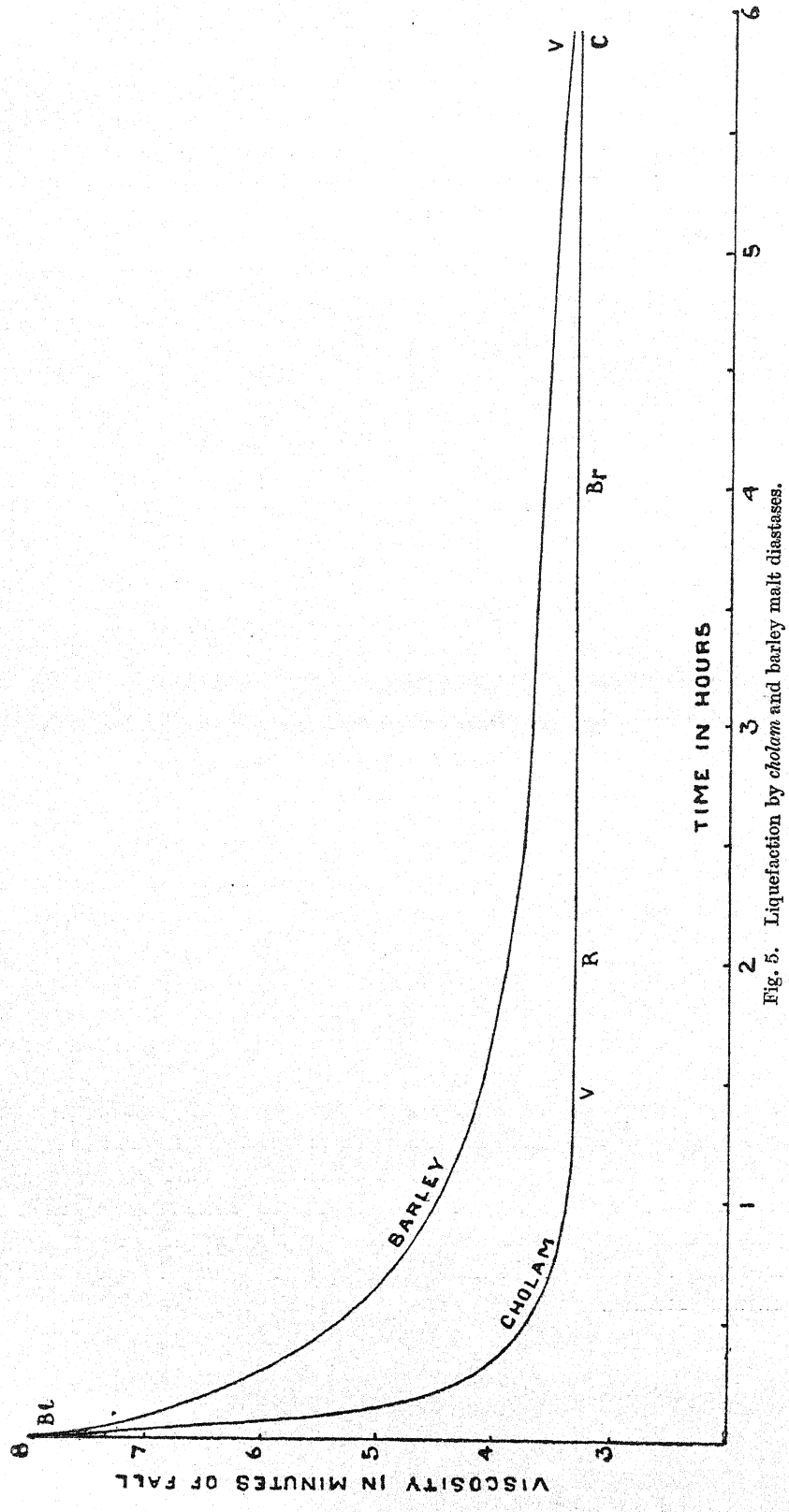


Fig. 5. Liquefaction by *cholam* and barley malt diastases.

THE SACCHARIFYING ACTIVITY OF *CHOLAM* AND BARLEY MALT DIASTASES.

Experiment (7). Saccharifying activity of cholam *and barley malt diastases at different pH ranges.*

0·02 grm. each of precipitated *cholam* and barley diastases was separately dissolved in 20 c.c. of water and filtered, and one c.c. of the filtered solution added to portions of 50 c.c. of 2 per cent. starch solution taken in 100 c.c. flasks. The starch solution was buffered to the required pH by the addition of 20 c.c. of McIlvaine's Buffer. The mixture was digested for one hour at 30°C., after which further action was stopped by the addition of 20 c.c. of $N/10$ sodium hydroxide solution, the volume made up to 100 c.c. and an aliquot taken for reducing power determination. The results obtained are shown in Fig. 6 and yield the following inferences.

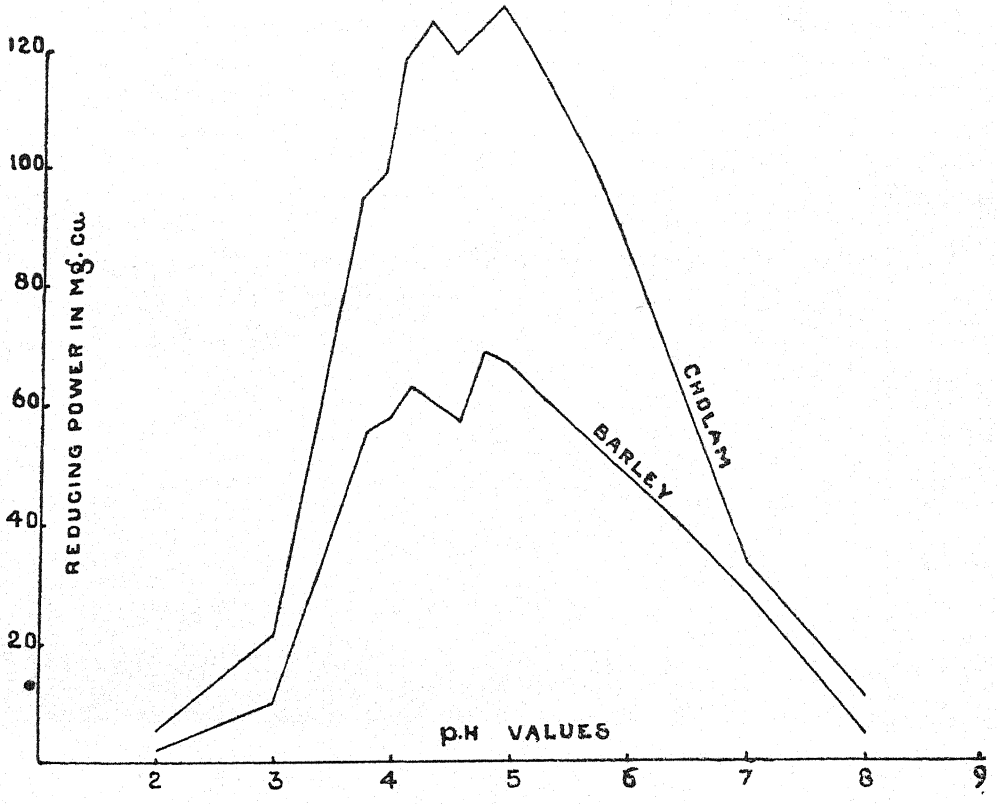


Fig. 6. pH and saccharification.

(1) The pH-saccharification curves for both *cholam* and barley are similar, in that they both show maximum activity between pH 4 and 5, and moderate activity between pH 3·0 to 4·0 and 5·0 to 6·0. Outside the range of pH 3·0 to 6·0 the saccharifying activity becomes negligible.

(2) Both the curves are double-topped ones, showing two maxima, a lower one at pH 4·2-4·4, and a higher one at pH 4·8-5·0. The lower maximum of pH 4·2 is also the optimum pH for maltase activity, as noted in Part I, but the increased saccharification at the above pH cannot be due to maltase action, since it has already been shown that alcohol precipitated diastase does not contain maltase, and further that maltase, in the amounts present in malt, does not show measurable activity in one hour.

Experiment (8). Saccharifying activity of cholam *and barley malt diastases at different temperatures.*

0·02 grm. of each enzyme was dissolved in 20 c.c. of water and filtered. Two c.c. of the filtrate were added to 50 c.c. of 2 per cent. starch, buffered to pH 4·8 by the addition of 20 c.c. of buffer. The flask was kept at the required temperature for one hour, after which 20 c.c. of $N/10$ sodium hydroxide were added to stop further action, the volume made up to 100 c.c. and an aliquot taken for cupric reduction.

The results represented graphically in Fig. 7 show that the saccharifying activity of both *cholam* and barley diastases have their optimum at the range of 45-50° C. and that above 60° C. the saccharification rapidly falls off, the fall being more rapid with barley than with *cholam*.

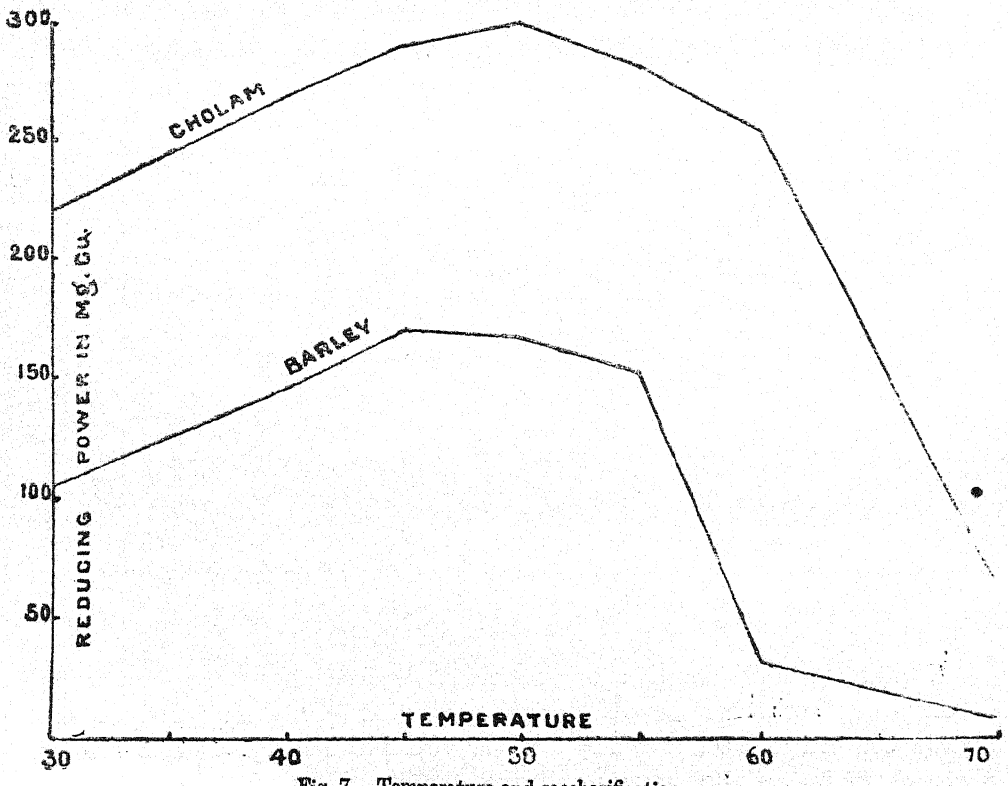


Fig. 7. Temperature and saccharification.

Experiment (9). Influence of concentration of enzyme on the saccharifying activity of cholam *and barley malt diastases.*

0·02 grm. of each *cholam* and barley diastases was separately dissolved in 20 c.c. of water and filtered. To 50 c.c. of 2 per cent. starch solution and 20 c.c. of 4·8 pH buffer, 0·5, 1·0, 1·5, 2·0, 3·0, 4·0, and 5·0 c.c. of enzyme solution were added. Digestion proceeded for one hour at 30° C. and the rest of the procedure was as in the previous experiments.

TABLE VI.

Amount of enzyme	Reducing power in mgrm. Cu	
	Cholam	Barley
0·5	64	28
1·0	126	56
1·5	186	82
2·0	238	108
3·0	356	160
4·0	432	208
5·0	504	264

The figures show that:—(1) At the early stages, there is an almost linear relationship between the amount of enzyme and reducing power produced, in accordance with Kjeldahl's " Law of proportionality". (2) This linear relationship is better maintained in the case of barley than in the case of *cholam*, where the curve tends to slope down even in the early stages.

Experiment (10). Influence of concentration of substrate on saccharification by cholam *and barley malt diastases.*

0·02 grm. of each *cholam* and barley diastases was dissolved in 20 c.c. of water, and 2 c.c. of the filtered extract added to 50 c.c. of starch solution of different concentrations, one per cent., 2 per cent., 3 per cent., 4 per cent. and 5 per cent., buffered with 20 c.c. of pH 4·8 buffer. The flasks were kept at 45° C. for one hour. Other details were as usual.

TABLE VII.

Concentration of starch per cent.	Reducing power in mgrm. Cu	
	Cholam	Barley
1	190	99
2	285	107
3	330	115
4	365	120
5	400	124

The figures show a marked difference between *cholam* and barley diastases in regard to the influence of concentration of the substrate on the degree of saccharification, as measured by total reducing power. In the case of *cholam* diastase, the amount of substrate exerts great influence and the reducing power increases from 190 mgrm. at one per cent. starch concentration to 400 mgrm. copper at 5 per cent. starch concentration, whereas in the case of barley, increasing concentrations of substrate exert little influence and the reducing power increases only from 99 mgrm. copper at one per cent. starch concentration to 124 mgrm. copper at 5 per cent. concentration. This is an interesting point of difference between the two diastases, an examination of which might throw light on the differences in their composition, and is again reverted to at a later stage.

Experiment (11). Temperature of destruction of the saccharifying power of cholam *and barley malt diastases.*

0·02 grm. of each enzyme was dissolved in 30 c.c. of water, and 4 c.c. portions of the filtered extract were kept at the temperatures noted below for 15 minutes, after which they were cooled. Two c.c. of the enzyme, thus treated, were added to 50 c.c. of 2 per cent. starch solution and 20 c.c. of pH 4·8 buffer, and kept for one hour at 30°C. Other details were as usual.

TABLE VIII.

Temperature of heating (15 mins.)	Reducing power in mgrm. Cu	
	Cholam	Barley
°C.		
30	148	84
60	80	20
65	52	15
70	12	10
75	6	5
80	3	2
85	*nil*	*nil*

The figures show that the saccharifying power, in the case of both *cholam* and barley, is greatly diminished by heating for 15 minutes above 65°C., but it is found

difficult to remove the last traces of reducing power. Heating at 85°C. for 15 minutes seems to destroy the saccharifying power almost completely.

Experiment (*12*). *Effect of other substances on the saccharifying activity of* cholam *and barley malt diastases.*

To 50 c.c. of 2 per cent. starch solution and 10 c.c. of pH 4·8 buffer, the following substances in the amounts noted in the table below were added, and then one c.c. of an enzyme solution prepared by dissolving 0·02 grm. of *cholam* diastase or 0·04 grm. of barley diastase in 25 c.c. and filtering. The reaction was allowed to proceed at 30°C. for one hour and determinations were made as usual. Controls were run simultaneously with the addition of toluene only.

TABLE IX.

Substance added	Amount	*Cholam* malt diastase (Reducing power of 10 c.c. in mgrm. Cu)			Barley diastase (Reducing power of 10 c.c. in mgrm. Cu)		
		Cont.	Exp.	Diff.	Cont.	Exp.	Diff.
(1) Ethyl alcohol	10 c.c.	87	73	—14	75	57	—18
(2) Glycol	,,	87	71	—16	75	65	—10
(3) Glycerol	,,	87	67	—20	75	68	—7
(4) Mannitol	2 grm.	87	84	—3	not examined		
(5) Glycine	1 grm.	104	138	+34	79	93	+14
(6) Asparagine	,,	104	108	+4	79	82	+3
(7) Aspartic acid	,,	104	25	—79	79	12	—67
(8) Pepsin	0·1 grm.	75	83	+8	90	91	+1
(9) Trypsin	,,	75	81	+6	90	94	+4
(10) Emulsin	,,	75	40	—35	90	45	—45
(11) Phenol	1 grm.	82	53	—29	77	5	—72
(12) Salicylic acid	0·5 grm.	82	0	—82	77	0	—77
(13) Potassium cyanide	0·1 grm.	82	0	—82	77	8	—69
(14) Potassium sulphocyanide	,,	82	83	+1	not examined		

The figures show that :—

(1) The influence of the extraneous substances tried is similar in the case of both barley and *cholam*.

(2) The alcohols have a depressing effect, whose intensity increases in the order, ethyl alcohol, glycol and glycerol, *i.e.*, with the number of hydroxyl groups in the molecule, but mannitol seems to be an exception. A similar behaviour was noted in the case of maltase also (p. 490), but the depressing effect of glycerol on diastase is much less than on maltase.

(3) The amino-acids have an accelerating effect, chiefly glycine. Aspartic acid, however, has a serious depressing effect, probably due to the increased acidity produced.

(4) Among other enzymes, pepsin and trypsin have a beneficial influence, while emulsin has a great depressing influence on saccharifying activity, which contrasts strongly with its absence of effect on maltase. Pringsheim and co-workers [1925] prepared from malt an amylase free from maltase by using glycerol to suppress maltase, but they failed to obtain an amylase-free maltase. It is worth while examining whether the suppressing influence of emulsin on diastase could be utilised for obtaining an amylase-free maltase.

(5) Among other substances tried, phenol, salicylic acid and potassium cyanide destroy saccharogenic action, while potassium sulphocyanide has no effect.

Experiment (13). A comparison of the liquefying and saccharifying activities of cholam *and barley malt diastases.*

Such relative amounts of *cholam* and barley malt diastases were taken as would produce approximately the same initial reducing power. 0·015 grm. of *cholam* diastase and 0·03 grm. of barley diastase were dissolved separately in 10 c.c. of water and 5 c.c. of the filtered extract were added to 200 c.c. of 2 per cent. starch, buffered to pH 4·8. Digestion proceeded at 30°C. and aliquots of 10 c.c. were taken every half hour in the beginning and later at intervals for reducing power determination. The iodine-colour change was also noted by removing drops of the solution from time to time and testing with a dilute solution of iodine. The results are shown in Table X and in Fig. 8.

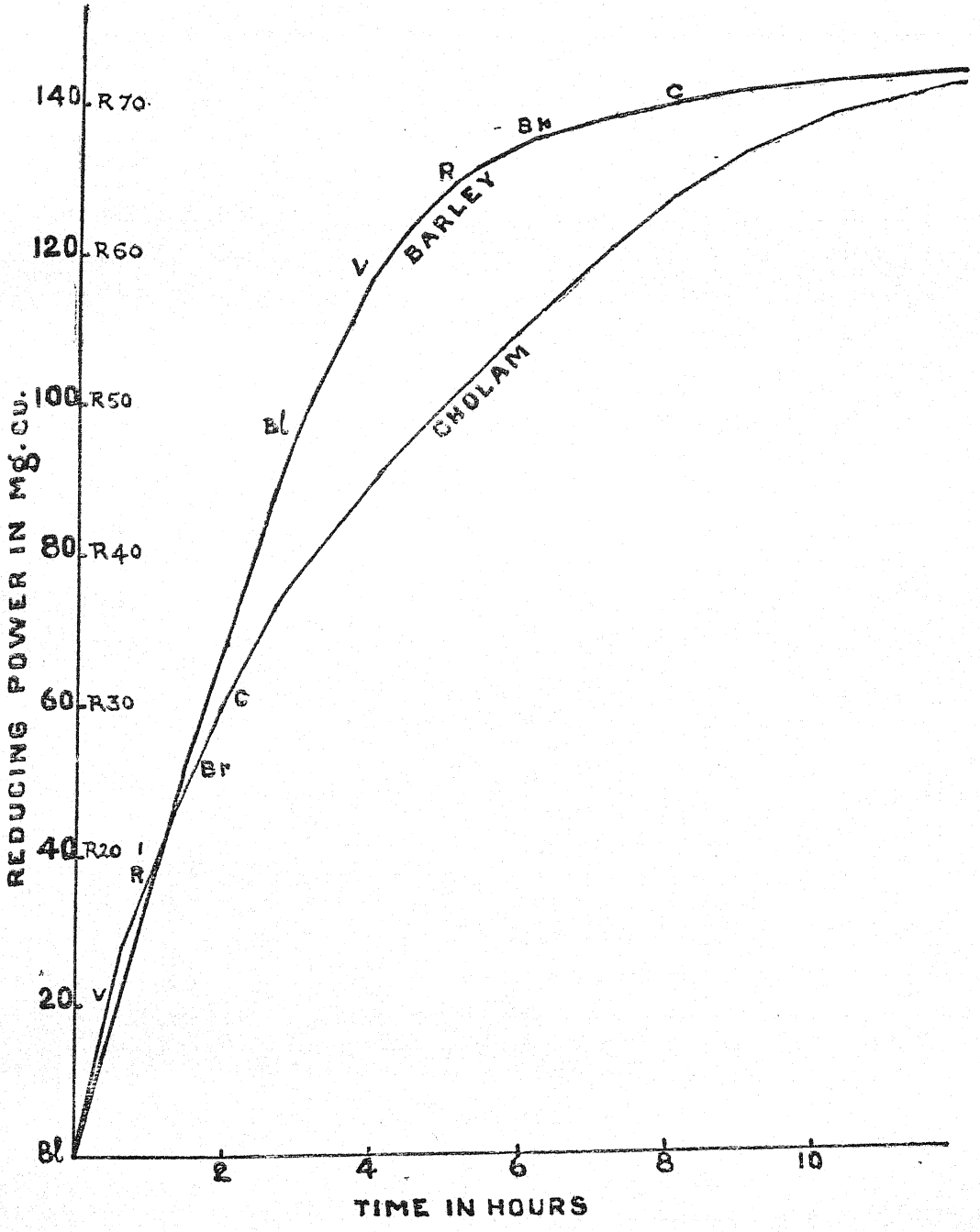


Fig. 8. Velocity of action of *cholam* and barley precipitated diastases.

TABLE X.*

Time in hours	*Cholam* malt diastase			Barley malt diastase		
	Iodine colour	Reducing power of 10 c.c. in mgrm. Cu	*R* (per cent. of apparent maltose)	Iodine colour	Reducing power of 10 c.c in mgrm. Cu	*R* (per cent. of apparent maltose)
(Start)						
0	Blue	*nil*	*nil*	Blue	*nil*	*nil*
½	Violet	22	11·0	,,	18	9·0
1	Red	37	18·5	,,	36	18·0
1½	Light brown	51	25·5	,,	53	26·5
2	Colourless	62	31·0	,,	68	34·0
2½	..	70	35·0	,,	83	41·5
3	..	78	39·0	,,	98	49·0
4	..	91	45·5	Bluish violet	118	59·0
5	..	102	51·0	Red	130	65·0
6	..	111	55·5	Brown	136	68·0
7	..	120	60·0	Light brown	138	69·0
8	..	128	64·0	Colourless	141	70·5
9	..	134	67·0	..	143	71·5
10	..	139	68·5	..	144	72·0
30	..	157	78·5	..	156	78·0

* The values of "*R*" in this and succeeding tables refer to the total reducing power of the solution expressed as percentage of maltose on starch taken.

The figures show that :—

(1) *Cholam* malt diastase is a much more powerful liquefier than barley malt diastase ; the iodine colour turns violet in ½ hr. with *cholam*, while with barley the change required about 4 hours.

(2) In the earliest stages (first hour), both the diastases produce about the same reducing power. But, while the action of barley diastase continues at about the same velocity till about 60 per cent. of the starch is converted into "apparent maltose" (*R* 60), the velocity of *cholam* malt diastase slows down progressively

from the very beginning. It will be noted from Fig. 8 that the curve relating to saccharification with time is almost linear for a longer time and for a longer range of reducing power in the case of barley (60 per cent. conversion) whereas in the case of *cholam*, the curve is linear for only a very short period (10 per cent. apparent maltose) and then begins to slope down.

(3) In the case of both *cholam* and barley curves, the linear relationship between saccharification and time holds good as long as the iodine colour remains blue, and the "sloping down" of the curve begins after the iodine colour changes to violet. In the case of *cholam*, the violet stage is reached early ($\frac{1}{2}$ hour at about *R* 10) and hence the saccharification curve begins to slope down from the earliest stages, whereas in the case of barley, the violet stage is reached only after a comparatively much longer time (4 hours at about *R* 60) and the saccharification curve is also linear till this stage.

(4) It has to be noted further that in the case of barley, when the reducing power represents about 65 per cent. conversion of starch into "apparent maltose" (*R* 65), further action becomes very slow, and the curve shows a sudden bend, and becomes almost horizontal. In the case of *cholam*, though the curve begins to flatten out from the very beginning, still the decrease in the velocity of action occurs only gradually, so that the curve goes steadily up: and finally, at the stage when the reducing power represents about 70 to 75 per cent. conversion (*R* 70), the *cholam* and barley curves approach one another. After 30 hours, when the action reaches equilibrium, the final value obtained is the same in both the cases (about *R* 80).

Experiment (*14*). *Influence of concentration of substrate on the velocity of action of* cholam *and barley diastases.*

It was already noted (p. 499) that concentration of substrate has great influence on the saccharification of *cholam* diastase, whereas it had little influence on that of barley. It seemed worth-while to investigate the matter a little further by comparing the velocities of liquefaction and saccharification of *cholam* and barley diastases at two different concentrations of starch.

For this purpose, 0·01 grm. of *cholam* diastase and 0·02 grm. of barley diastase were separately dissolved in 20 c.c. of water, filtered, and 5 c.c. portions of the filtrate added to 200 c.c. of 2 per cent. and 4 per cent. starch solutions, buffered to pH 4·8. Aliquots of 10 c.c. were taken for cupric reduction. The figures obtained are shown in Table XI and also in Figs. 9 and 10.

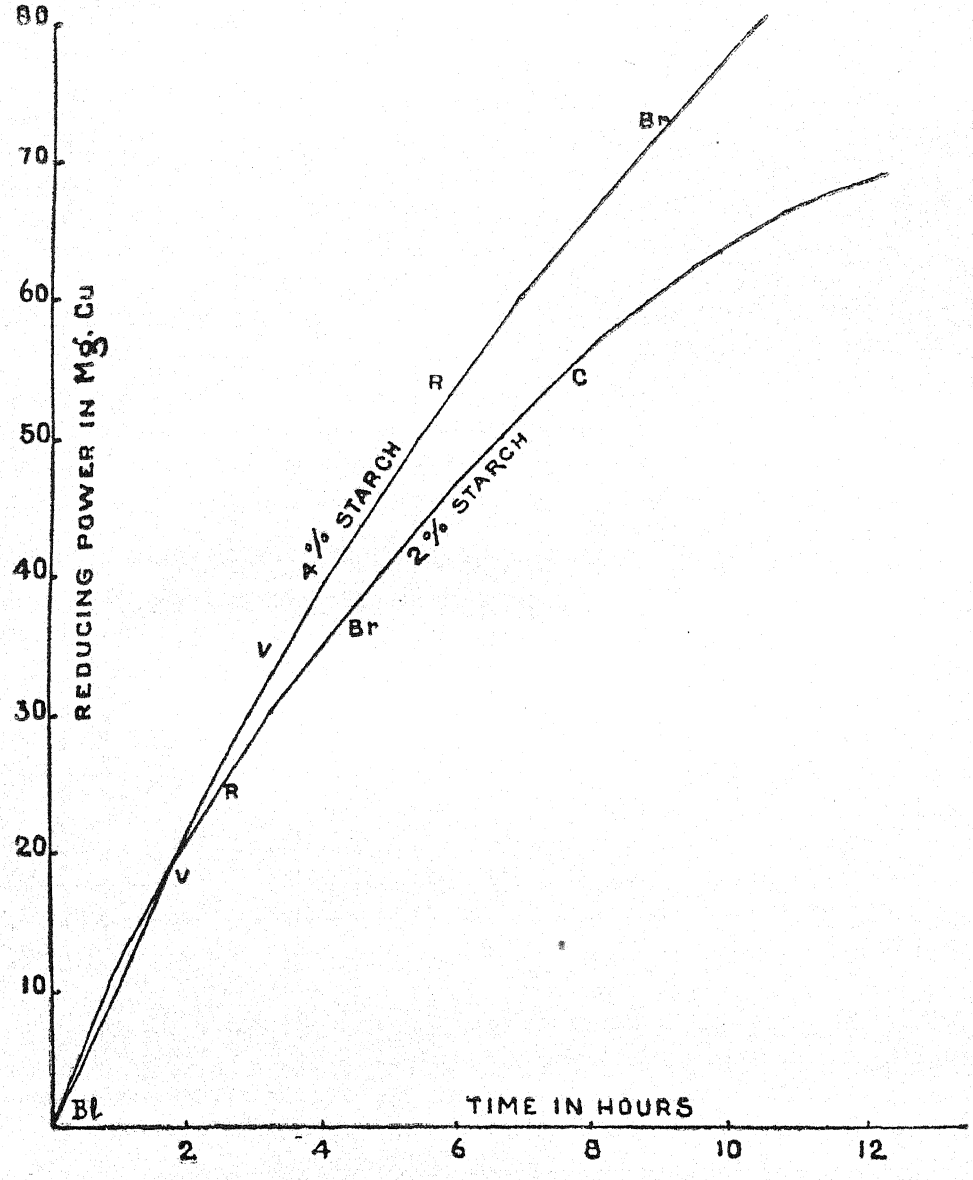


Fig. 9. Effect of concentration of substrate on *cholam* diastase.

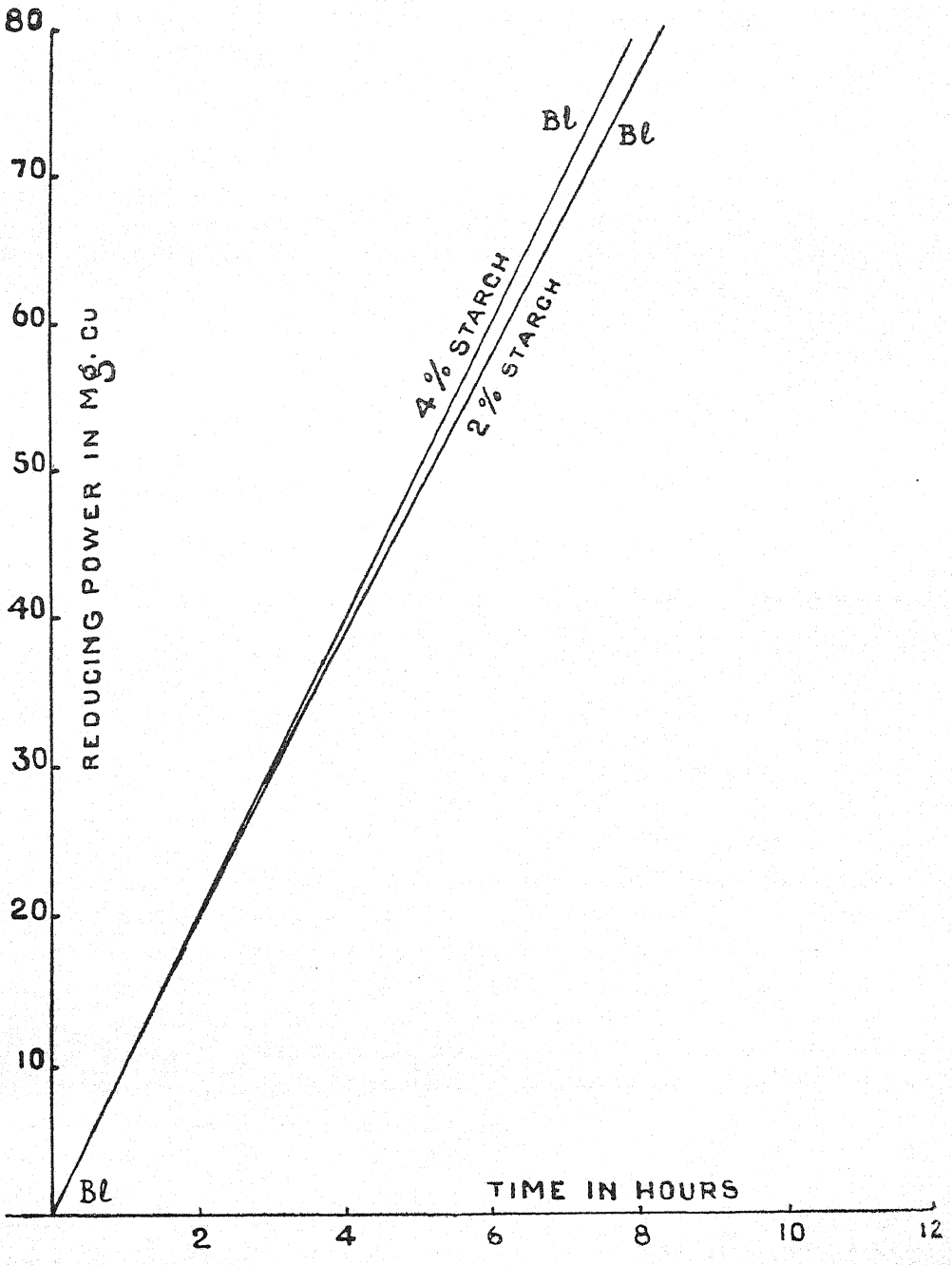


Fig. 10. Effect of concentration of substrate on barley diastase.

TABLE XI.

Time in hours	*Cholam* malt diastase				Barley malt diastase			
	2 per cent. starch		4 per cent. starch		2 per cent. starch		4 per cent. starch	
	R. P. of 10 c.c. mgrms. Cu	Iodine colour	R. P. of 10 c.c. mgrms. Cu	Iodine colour	R. P. of 10 c.c. mgrms. Cu	Iodine colour	R. P. of 10 c.c. mgrms. Cu	Iodine colour
½	6	Blue	6	Blue	5	Blue	5	Blue
1	10	,,	10	,,	10	,,	10	,,
1½	15	,,	15	,,	15	,,	15	,,
2	19	Violet	20	,,	20	,,	20	,,
2½	23	,,	25	,,	25	,,	25	,,
3	27	Red	30	,,	30	,,	30	,,
4	34	Brown	39	Violet	39	,,	40	,,
5	40	,,	47	,,	49	,,	50	,,
6	46	Light brown	54	Red	58	,,	59	,,
7	52	,,	60	,,	67	,,	69	,,
8	57	Colourless	66	,,	76	,,	78	,,
9	61	,,	72	Brown	84	,,	87	,,
10	64	,,	78	,,	93	,,	95	,,
24	110	,,	146	Colourless	144	Brown	212	Violet

From the above table and figures, it may be noted :—

(1) The saccharifying power is almost linear, so long as the iodine-blue colour lasts, in the case of both *cholam* and barley diastases, for different concentrations of starch.

(2) As long as the iodine colour remains blue, further increase of concentration of substrate has no effect on the saccharifying power, which under the above conditions becomes a function of the quantity of the enzyme and not of the substrate. Thus, both 2 per cent. and 4 per cent. concentrations of starch give identical curves till the iodine colour of the 2 per cent. concentration of starch turns violet.

(3) After the iodine colour changes to violet, concentration of substrate has a great influence on the rate of saccharification. Thus, the rate of saccharification falls off in the case of (*cholam*—2 per cent. starch) after 2 hours when the violet colour is reached, while in the case of (*cholam*—4 per cent. starch) the saccharification curve continues to be linear up to 4 hours, when only the iodine colour changes to violet. In the case of barley, the effect of concentration of substrate is not apparent, since the iodine colour remains blue for a long period (10 hours for 2 per cent. starch and 20 hours for 4 per cent. starch), and hence, both (barley—2 per cent. starch) and (barley—4 per cent. starch) show almost identical curves up to 10 hours. But even here, after (barley—2 per cent. starch)

crosses the iodine-violet stage, there is marked difference between the results of 2 per cent. and 4 per cent. starches. Thus, after 24 hours, 2 per cent. starch shows a reducing power of 144 mgrms. of copper while 4 per cent. starch shows 212 mgrms.

(4) It has already been noted that Kjeldahl's "Law of proportionality", which applies to barley diastase up to *R* 40 (cupric reduction expressed as maltose percentage on starch), does not apply to *cholam* diastase above *R* 10. The results now obtained would suggest that for a proper comparison of the strengths of diastases, either from the same source or from different sources (*e.g.*, barley and *cholam*), care should be taken to stop the comparative reactions while still the substrates are all within the iodine-violet stage, as only under these conditions, the respective activities of the enzymes are proportional to their cupric reducing powers. Kjeldahl's Law embodies this general rule with special reference to barley malt diastase, where the iodine-violet colour is obtained only at about *R* 50.

(5) Concentration of starch appears to have a great influence on the saccharification of *cholam* and not of barley diastase, since the iodine-violet colour is reached quickly in the case of *cholam* and only at a late stage in the case of barley.

(6) A careful consideration of the data, especially in regard to *cholam* diastase, tends to show that the decomposition of starch occurs in a "latitudinal" way, by a sequence of regular and well defined stages; in other words, the whole body of the substrate, irrespective of its concentration, has to be degraded to the same level, before the enzyme attempts a further degradation to a lower level. This will be apparent from the following figures taken from Table XV :—

	Iodine-violet		Iodine-red		Iodine-brown	
	Time	*R*	Time	*R*	Time	*R*
2 per cent. starch	2 hrs.	19 mgrms.	3 hrs.	27 mgrms.	5 hrs.	40 mgrms.
4 „ „	4 „	39 „	6 „	54 „	10 „	78 „

Experiment (*15*). *Effect of preliminary heating to high temperature* (*60°C.*) *on the saccharification curves of* cholam *and barley amylases.*

It has been noticed by Norris and Viswanath [1923] and Viswanath and Suryanarayana [1925] that the saccharification curves of *cholam* malt diastase are similar both at high and at ordinary temperatures, while they are different in the case of barley. An attempt was made to verify this observation by the following experiment :—0·04 grm. of *cholam* diastase and 0·08 grm. of barley diastase were separately dissolved in 20 c.c. of water and filtered. 5 c.c. of the filtrate were added to 200 c.c. of 2 per cent. starch solution, buffered to pH 4·8; and the rest of the filtrate was heated to 60°C. for 15 minutes and then 5 c.c. of the heated enzyme added to another portion of 200 c.c. of 2 per cent. starch solution.

Aliquots of 10 c.c. were taken for reducing power determination. The figures obtained are shown in Table XII and in Figs. 11 and 12 :—

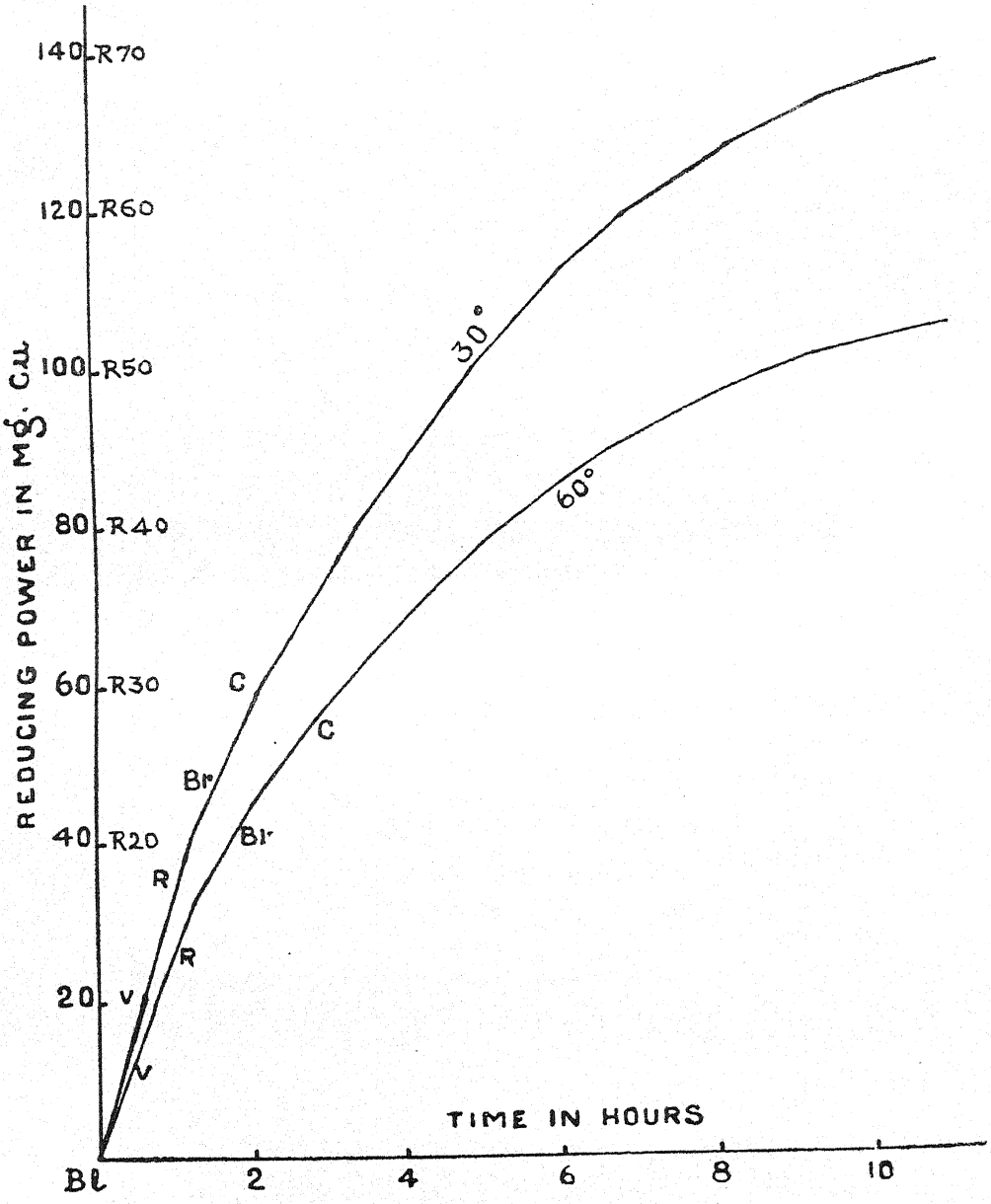


Fig. 11. Effect of preliminary heating on *cholam* diastase.

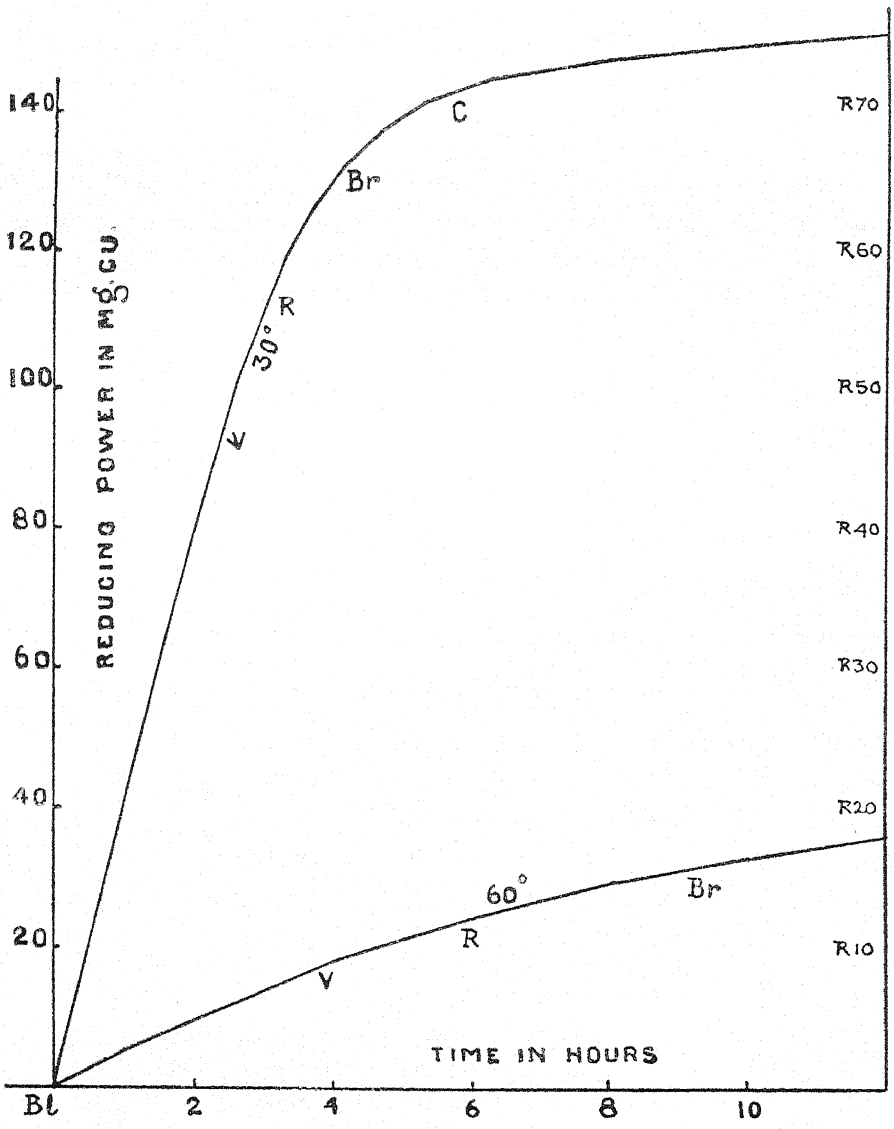


Fig. 12. Effect of preliminary heating on barley diastase.

(1) The table and figures confirm the observation of previous workers that the curves for *cholam* diastase are similar both at high and at ordinary temperatures, whereas those for barley show great difference.

(2) The saccharification curve of barley at high temperature (60°C.), in its general shape and also in its iodine-colour to reducing power relationship, resembles the curve for *cholam*. Thus, whereas at ordinary temperatures, the barley enzyme shows iodine violet colour only at a reducing power of *R* 50 (percentage of apparent maltose), the heated enzyme shows the iodine violet colour at a reducing power of about *R* 8 (percentage of apparent maltose) which is close to that of *cholam*, both at ordinary and at high temperature *R* 8-9 (apparent maltose). Thus pre-heated barley enzyme (60° C.) appears to decompose starch in a similar way to the *cholam* enzyme.

TABLE XII.

Time in hours	*Cholam* malt diastase						Barley malt diastase					
	Unheated			Heated to 60° C.			Unheated			Heated to 60° C.		
	R. P. of 10 c.c. mgrm. Cu	*R*	Iodine colour	*R. P.* of 10 c.c. mgrm. Cu	*R*	Iodine colour	*R. P.* of 10 c.c. mgrm. Cu	*R*	Iodine colour	*R. P.* of 10 c.c. mgrm. Cu	*R*	Iodine colour
½	19	9·5	Violet	15	7·5	Violet	21	10·5	Blue	3	1·5	Blue
1	35	17·5	Red	27	13·5	Red	41	20·5	,,	5	2·5	,,
1½	48	24·0	Brown	37	18·5	Brown	60	30·0	,,	7	3·5	,,
2	60	30	Colour-less	45	22·5	Light brown	81	40·5	,,	9	4·5	,,
2½	69	34·5	,,	53	26·5	,,	98	49·0	Purple	11	5·5	,,
3	76	38·0	,,	59	29·5	Colour-less	114	57	Violet	13	6·5	,,
4	90	45·0	,,	69	34·5	,,	130	65	Red	17	8·5	Violet
5	102	51·0	,,	77	38·5	,,	140	70	Brown	20	10·5	,,
6	112	56·0	,,	85	42·5	,,	144	72	Colour-less	23	11·5	Red
7	120	60·0	,,	90	45·0	,,	147	73·5	,,	26	13·0	,,
8	127	63·5	,,	94	47·0	,,	147	73·5	,,	28	14·0	,,
9	132	66·0	,,	97	48·5	,,	148	74·0	,,	30	15·0	Brown
10	137	68·5	,,	100	50·0	,,	..	..	..	..	..	..
24	..	..	..	..	..	..	156	78·0	Colour-less	56	28·0	Colour-less

Experiment (16). Formation of glucose at high temperatures by the action of cholam *and barley malt diastases.*

It has been noticed by Ling and Nanji [1923] and others that when barley malt diastase is subjected to a preliminary heating above 60° C. glucose could be observed among the products of hydrolysis. With a view to test this observation in regard to both *cholam* and barley malt diastases, experiments were made on the lines of Experiment No. 15, and 20 c.c. portions were taken at intervals from the non-heated and pre-heated enzyme flasks for the determination of glucosazone by heating with phenyl-hydrazine and acetic acid, according to the procedure already standardised (p. 480). It was found that:—

(1) In the cases of *cholam* and barley malt diastases, not subjected to preliminary heating, no glucosazone was formed; but it was noticed that a great deal of maltose was formed in the case of barley diastase, while only a small amount was formed in the case of *cholam* diastase.

(2) When the two diastases were subjected to preliminary heating just above 60° C. for 10-15 minutes, and then reacted with starch for about a week in presence of toluene, glucosazone could be isolated in the cases of both *cholam* and barley diastases. But the glucose could be identified earlier and obtained in larger quantities in the case of *cholam* than with barley diastase.

(3) The formation of glucose cannot be due to the action of maltase, since it has already been seen (p. 484) that alcohol precipitated diastase does not contain maltase, and moreover it has been shown that maltase in solution is inactivated by heating at 60° C. for 15 minutes (p. 492).

(4) This question of glucose formation at higher temperatures will be taken up for consideration in the next part.

Summary.

(1) A comparative study of *cholam* and barley malt diastases has been made in regard to their liquefying and saccharifying properties, with a view to examine whether any characteristic differences exist in regard to their mode of action on starch.

(2) *Cholam* malt diastase is a much more powerful liquefier of starch than barley malt diastase, as evidenced by the quicker fall of viscosity and the shorter time required for the change of iodine colour to violet. It has got two pH optima (when the liquefying power is measured by fall of viscosity) at pH 4·8 and 6·0, and an optimum temperature of 50° C., as compared with one pH optimum of pH 5·0 and an optimum temperature of 45° C. for barley malt diastase. The liquefying power of *cholam* diastase is destroyed by heating at 80° C. in solution for 15

minutes, while a temperature of 75° C. for 15 minutes is sufficient to inactivate the liquefying power of barley.

(3) In the case of both *cholam* and barley malt diastases, there is a close relationship between the viscosity fall and iodine colour change methods for the determination of the rate of liquefaction. Immediately after the addition of the enzyme, there is a rapid fall of viscosity, and most of the total fall of viscosity is completed while the iodine colour still remains blue, suggesting that in both cases, the first change is probably a depolymerisation of starch into simpler molecules which continue to give the blue colour with iodine. The second stage of hydrolysis begins when the iodine colour changes to violet and red; at this stage, the viscosity curve shows a sharp bend and becomes almost horizontal, showing little further fall in viscosity.

(4) In regard to their saccharifying powers at different pH ranges, both the diastases show double-topped curves, having a lower maximum at pH 4·2—4·4 and a higher maximum at pH 4·8—5·0. It is shown that the lower maximum, though it coincides with the optimum for maltase activity, could not be due to it. Both the diastases have an optimum temperature of 45°—50°C., and are inactivated by heating in solution to 85°C. for 15 minutes.

(5) In the case of barley malt diastase, the cupric reducing power is proportional to concentration of the enzyme up to *R* 40, in accordance with Kjeldahl's "law of proportionality", whereas in the case of *cholam*, the proportionality is limited to *R* 10.

(6) The influence of other substances on saccharification was similar in the case of both *cholam* and barley diastases. Ethyl alcohol, glycol and glycerol exert an increasingly depressing influence; glycine and asparagine have an accelerating effect, while aspartic acid depresses. Pepsin and trypsin have a beneficial influence, while emulsin greatly depressed saccharification. Phenol, salicylic acid and potassium cyanide inactivate the enzyme, while potassium sulphocyanide has no influence.

(7) The amount of substrate exerts a much greater influence on the saccharifying activity of *cholam* diastase than on barley diastase; thus, the reducing power was doubled in the case of *cholam* diastase by increasing the starch concentration from one per cent. to 4 per cent. while in the case of barley, the increase was only 20 per cent. A detailed examination of this question showed that the influence of substrate began after the iodine colour changed to violet. Within the iodine-violet limit, saccharification was a function of enzyme concentration only and not of substrate. It is suggested that comparisons of diastatic powers of enzymes, either from the same source or from different sources, by means of reducing power, should be limited to within the iodine-violet range.

(8) It is shown that the saccharification curves are similar for *cholam* both at ordinary and at higher temperatures (60°C.), while in the case of barley, they are widely different. The curve for barley at higher temperatures (60°C.) resembles the curve for *cholam*.

(9) When *cholam* or barley malt diastase is heated in solution to 60°C. and then reacted with starch, glucose is one of the ultimate products of hydrolysis.

III. On the two components of amylase.

The experiments reported in Part II served to confirm the observations of previous workers regarding the greater liquefying and lesser saccharifying power of *cholam* malt diastase as compared with the barley enzyme and further, to throw additional light relative to differences in the mechanism of disintegration of starch by the two enzymes, as shown by the time-velocity curves of the diastases and the variation in the relationship between iodine reaction and saccharification in the two cases, the differential effects of substrate concentration on the two enzymes, their differential behaviour at ordinary and at higher temperatures, the greater abundance of glucose formation by the action on starch of *cholam* amylase previously heated to 60°C. for a few minutes, etc. It has been already observed how previous workers have tried to explain some of these differences on the basis of the two-enzyme theory of amylase, but so far no satisfactory experimental evidence has been offered in this connection, say by the isolation and study of the two components separated from each other*.

Probably the most continuous piece of work on this aspect of amylase is that of Ohlsson [1922, 1926, 1930] who appears to have confined himself mainly to barley malt diastase. He claims to have separated barley malt diastase into two fractions—one a powerful liquefier, called dextrinogenase, and the other a powerful saccharifier, known as saccharogenase—by the differential effects of pH and temperature. He finds that when diastase is kept at pH 3·3 for 15 minutes at ice-box temperature, the liquefying component is mostly lost, leaving behind the saccharogenic power about 75 per cent. active ; while, by keeping the diastase in solution at 75 C. for 15 minutes (at a pH of 6 to 7) the saccharifying component is mostly destroyed, leaving behind the liquefying component about 75 per cent. active. It was thought that a verification of Ohlsson's experiments, with reference to other diastases like *cholam*, might throw further light on the heterogeneity or homogeneity of amylase. The

* Since the conclusion of these experiments in October 1931, Waldschmidt-Leitz, Reichel and Purr [1932] have reported the successful separation of α and β-amylases in malt by adsorption on alumina Cγ and the use of amylo-kinase. Holmberg [1933] has effected their selective adsorption by the use of starch and 40—50 per cent. alcohol. Iyengar, Narayana, Sastri and Srinivasayya [1933] have also reported the successful adsorption of the liquefying component using amylopection.

following experiments are typical of several similar ones carried out and illustrate the conclusions arrived at.

Experiment (1). Preparation of "saccharogenase" from barley malt diastase.

0·10 grm. of barley malt diastase was dissolved in 20 c.c. of water and filtered. Four c.c. portions, taken in test-tubes, were brought to pH 3·2 by the addition of 4 c.c. of McIlvaine's Buffer (3 c.c. *M*/10 citric acid and 1 c.c. *N*/5 disodium phosphate). The test-tubes were kept in ice for varying intervals of time, as noted in Table I below, after which they were brought to pH 4·9 by adding 2 c.c. of *N*/5 disodium phosphate, making up the total volume to 10 c.c. Five c.c. of the resulting solution, corresponding to 2 c.c. of the original diastase solution, were then added to 200 c.c. of 2 per cent. starch solution, buffered to pH 5·0 and kept at 30°C. Ten c.c. aliquots were taken out at intervals for reducing power determination.

TABLE I.

pH 3·2 and 0°C.	*R. P.* after 1 hour mgrm. Cu	*R. P.* after 2 hours mgrm. Cu	Liquefying power— time for iodine-red change
1. No treatment	22	41	6 hours.
2. 5 min. treatment	20	38	48 ,,
3. 15 min. treatment	20	39	48 ,,
4. 30 min. treatment	20	38	48 ,,

The experiment was repeated a number of times giving similar results, showing that :—

(1) When barley malt diastase is kept at pH 3·2 and 0°C. for more than 5 minutes, it loses a definite percentage of its initial reducing power (from 22 to 20 mgrms.).

(2) That this fall is not due to the destructive effect of pH on the enzyme is shown by the fact that continued treatment over 5 minutes, does not cause any further fall in reducing power ; in fact, the remaining reducing power is stable at pH 3·2 and 0°C.

(3) The liquefying power of barley malt diastase, as measured by the time taken for the change of iodine colour to red (which is normally poor as compared with *cholam*), is almost wholly lost, by keeping the enzyme solution under the above conditions (from 6 hours, the time for iodine-red changes to 48 hours).

(4) The definite percentage fall in reducing power, noted in para. (1) above, (2 mgrms. out of 22 mgrms.) may be associated with the weak liquefying power of barley, inasmuch as both of them are simultaneously lost under the above conditions.

Experiment (2). *Preparation of "dextrinogenase" from barley malt diastase.*

Ohlsson has recommended heating diastase at pH 6 to 7 to 70°C. for 15 minutes, to obtain a powerful liquefier (dextrinogenase) practically free from saccharogenic power.

When, however, barley malt diastase solutions were buffered to pH 6 to 7 by the addition of McIlvaine's Buffer and then heated to 73°C. or even to 65°C. the enzyme (both saccharifying and liquefying components) was completely destroyed in 10 minutes; that pH alone was not the destroying factor was shown by the fact that when the experiment was conducted at 30°C., the enzyme was found to be quite reactive. Experiments were therefore tried without the addition of buffer, by simply heating the diastase solution (in distilled water) to 60, 65 and 70°C., for 10 minutes; and it was found that this procedure yielded a preparation rich in liquefying power but possessing very little of saccharogenic power.

(*a*) *Effect of temperature.*—0·05 grm. of barley malt diastase was dissolved in 10 c.c. of water and filtered. The filtrate was kept at the required temperature for 15 minutes and 5 c.c. of the treated enzyme solution was added to 200 c.c. of 2 per cent. starch, buffered to pH 5·0. Digestion proceeded at 30°C. and 10 c.c. portions were taken for cupric reduction.

TABLE II(A).

Temperature of heating	R. P. of 10 c.c. after 1 hour in mgrm. Cu	Liquefying power—time for iodine-purple	
		Hours	Minutes
1. No treatment	60	3	30
2. 62°C.-63°C.	5	3	30
3. 65°C.	5	3	45
4. 70°C.	4	4	0
5. 75°C.	3	6	30

The above figures show that when barley malt diastase is heated at 63°C. for 15 minutes most of the reducing power is lost, but the whole of the liquefying power is conserved. When, however, the heating is done at higher temperatures, especially above 65°C. a portion of the liquefying power also is lost. It would, therefore, appear that if the liquefying power is not to be destroyed, 62°-63°C. is a better temperature for obtaining the liquefying component than Ohlsson's 70°C.

(*b*) *Effect of period of heating.*—The conditions of the experiment were the same as in (2*a*). but the enzyme solutions were heated at 62°-63°C. for varying intervals of time.

TABLE II(B).

62°-63°C.	R. P. of 10 c.c. after 1 hour in mgrm. Cu	Liquefying power—time for iodine-purple	
		Hours	Minutes
1. No treatment	60	3	30
2. 15 min. heating	5	3	30
3. 30 „ „	5	3	40
4. 45 „ „	5	3	50

The figures show that :—

(1) When barley malt diastase is heated at 62°-63°C. for 15 minutes or more, the reducing power shows a great but definite percentage fall.

(2) That temperature effect is not the only factor causing the fall is shown by the fact that longer times of heating beyond 15 minutes do not increase the fall.

(3) The liquefying power is stable at the temperature (62°-63°C.), and the stability of the remaining reducing power under similar conditions would show that it is associated with the liquefying components of diastase.

Experiment (*3*). *Preparation of saccharogenase from* cholam *malt diastase.*

(*a*) *Effect of pH 3·2 and 0°C. on* cholam *diastase.*—The conditions and details of the experiment were the same as for barley (Expt. 1).

TABLE III(A).

pH 3·2 and 0°C.	R. P. of 10 c.c. after 1 hour in mgrm. Cu	Liquefying power—time for iodine-purple	
		Hours	Minutes
1. No treatment	34	0	45
2. 5 min. treatment	18	1	15
3. 15 „ „	12	2	0
4. 30 „ „	9	6	0
5. 45 „ „	9	10	0
6. 60 „ „	9	16	0

The above figures would show that in the case of *cholam*, the liquefying component is not destroyed completely by keeping the diastase solution at pH 3·2 for 15 minutes, as with barley; a longer time, preferably one hour, is necessary for a constant value to be obtained. At the end of one hour, the liquefying power had fallen from 45 min. to 16 hours, while the reducing power also showed a great fall from 34 to 9 mgrms. copper, but was constant at the latter value.

(*b*) *Effect of pH 3·2 and 30°C. on* cholam *diastase.*—In order to shorten the time of keeping at pH 3·2 an attempt was made to hasten the destruction of the liquefying component by raising the temperature of treatment from 0°C. to 30°C. The other details are the same as in Expt. (3*a*).

TABLE III(B).

pH 3·2 and 30°C.	R. P. of 10 c.c. after 1 hour in mgrm. Cu	Liquefying power—time for iodine-purple
1. No treatment	36	35 minutes
2. 5 min. treatment	9	12 hours
3. 15 „ „	9	18 „
4. 30 „ „	9	24 „

Hence in the case of *cholam* malt diastase, keeping the solution at pH 3·2 and 30°C. for 15—30 minutes seems to be a quicker way of destroying the liquefying component than keeping at 0°C. under which conditions a longer time, say 1 or 2 hours, is necessary to arrive at the same result.

(*c*) *Effect of pH 3·2 and 30°C. on barley saccharogenase.*—The next point that was examined was whether the higher temperature of 30°C. at pH 3·2 would preserve or destroy the saccharogenase of barley diastase. Experiments were repeated as in Expt. (1), but keeping the tubes at 30°C. instead of 0°C.

TABLE III(C).

pH 3·2 and 30°C.	R. P. of 10 c.c. after 1 hour in mgrm. Cu	Comparison figures for R. P. at pH 3·2 and 0°C.
1. No treatment	23	22
2. 5 min. treatment	11	20
3. 15 „ „	7	20
4. 30 „ „	4	20
5. 60 „ „	1	20

The above figures show that the saccharogenase of barley cannot withstand a temperature of 30°C. at pH 3·2 and is rapidly destroyed, while a temperature of 0°C. is able to preserve it at the above pH.

(1) Hence, for obtaining the saccharogenase component of *cholam* and barley diastases under comparable conditions, it is better to keep the diastase solution at pH 3·2 and 0°C. for say an hour or longer.

(2) A point of difference between barley and *cholam* malt diastases, evident from a comparison of Tables I and III(A), lies in the fact that at pH 3·2 and 0°C. most of the reducing power of barley is conserved (20 out of 22 mgrms. of copper), while only a fourth of the reducing power of *cholam* is conserved (9 out of 36 mgrms. of copper). This seems to be in opposition to Ohlsson's idea that the saccharogenic component of diastase is stable at 0°C. and pH 3·2.

(3) It may also be noted that corresponding to the lower liquefying power of barley, there is only a small loss of the reducing power (2 mgrms. out of 22 mgrms.), while, corresponding to the greater liquefying power of *cholam*, there is a greater loss (27 mgrms. out of 36 mgrms.) when both the solutions are kept at 0°C. and pH 3·2, suggesting that the falls of reducing power in the two cases are associated with the respective liquefying components of the two diastases.

Experiment (4). *Preparation of dextrinogenase from* cholam *malt diastase.* In the case of barley it was noted (Expt. 2*a*) that Ohlsson's temperature of 0°C. was too high and destroyed a portion of the liquefying component. The present experiment was therefore made to see whether a similar effect was observed in the case of *cholam*.

(*a*) *Effect of temperature.*—The details of the experiment are the same as in Expt. (2*a*); instead of 0·05 grm. of barley diastase 0·02 grm. of *cholam* diastase was taken.

TABLE IV(A).

Treatment	Liquefying power—time for iodine-purple
1. No treatment	25 min.
2. Heating at 62°-63° C. for 10 min.	25 „
3. „ 65° C. „	30 „
4. „ 70° C. „	1 hour 30 minutes

Hence, heating above 65°C. destroys a portion of the liquefying capacity, while heating at 62°-63°C. for 10 minutes preserves it.

(*h*) *Effect of period of heating.*—The details of the experiment are the same as in (2*b*), but taking 0·02 grm. of *cholam* diastase in place of barley diastase.

TABLE IV(B).

62°-63° C.	R. P. of 10 c.c. after 1 hour in mgrm. Cu	Liquefying power—time for iodine-purple
1. No treatment	36	25 min.
2. Heating for 10 min.	27	25 „
3. „ 30 „	27	30 „

It would, therefore, appear that:—

(1) For obtaining the liquefying component from *cholam* the diastase may be heated in solution to 62°-63°C. for 10—15 minutes.

(2) Under these conditions, there is a constant fraction of the original reducing power (27 mgrms. out of 36 mgrms.) which is stable and is probably associated with the liquefying power.

Experiment (5). *Comparison of the saccharogenase and dextrinogenase content of barley and* cholam *malt diastases.*

Amounts of *cholam* and barley diastases which gave equal initial reducing powers were taken for comparison. Details for the preparation of the components have already been given under Expts. 1—4.

TABLE V.

Experimental details	Barley diastase (16 mgrms.) R. P. in mgrms. Cu	*Cholam* diastase (9 mgrms.) R. P. in mgrms. Cu
1. Initial R. P. (untreated)	36	36
2. R. P. after treatment at 0°C. and pH 3·2, *i.e.* R. P. associated with saccharogenase.	33	9
3. By subtraction, R. P. associated with liquefying component.	3	27
4. Liquefying power as reciprocal of time taken for change of iodine colour to violet.	1/3·75 hr. *i.e.*, 4/15	1/0·42 hr. *i.e.*, 12/5
5. R. P. after heating at 62°-63°C. for 15 minutes, *i.e.*, R. P. associated with liquefying component.	3	27
6. R. P. of liquefying component divided by liquefying power, *i.e.* (5)/(4).	11	11

The figures given in the foregoing table reveal several interesting points :—

(1) In the case of barley malt diastase, most of the reducing power is preserved by keeping the diastase in solution at 0°C. and pH 3·2. There is a fall of 3 mgrms. only out of 36 mgrms. This fall, however, is significant and is probably associated with the small amount of liquefying power possessed by barley diastase.

(2) With *cholam* diastase, on the other hand, which possesses a high degree of liquefying power, the fall of reducing power when the solution is kept at 0°C. and pH 3·2, is much greater, *i.e.*, 27 mgrms. out of 36 mgrms. This appears to be contrary to Ohlsson's hypothesis that saccharogenase is stable at 0°C. and pH 3·2, and can be satisfactorily explained only by supposing that the fall in reducing power is associated with the greater liquefying power possessed by *cholam* malt diastase.

(3) Again, when barley malt diastase is heated at 62°-63°C. for 10—15 minutes, the reducing power falls from 36 mgrms. to 3 mgrms. and the liquefying power is almost wholly preserved. The remaining reducing power is significant, when we note that it cannot be still further lowered except at the loss of a corresponding fraction of the liquefying power (*cf.* Expt. 2*a*).

(4) When *cholam* malt diastase is heated at 62°-63°C. for 10—15 minutes, the reducing power falls from 36 mgrms. to 27 mgrms. and the liquefying power is wholly preserved. Here also the remaining reducing power seems to be closely associated with the liquefying power, since it cannot be lowered except with a corresponding loss of liquefying power (*cf.* Expt. 4*a*).

(5) Ohlsson's experiments seem to have been confined to barley malt diastase mainly. With this diastase, nearly 90 per cent. of the reducing power is preserved (33 mgrms. out of 36 mgrms.) at 0°C. and pH 3·2, and when heated to above 63°C. only a small amount of the original reducing power is left (3 out of 36), associated with the weak liquefying power of barley. In the light of this behaviour of barley diastase, Ohlsson's conclusions seem natural *viz.*, that diastase consists of two components, a liquefying one dextrinogenase possessing practically no saccharifying power, and a saccharifying component (saccharogenase) which possesses no liquefying power. But Ohlsson, even working with barley, has had to admit, after repeated trials, that he could not prepare a dextrinogenase free from saccharifying power or a saccharogenase completely devoid of liquefying power (as shown by iodine reaction).

(6) When Ohlsson's experiments are, however, repeated with diastases possessing higher liquefying power than barley, *e.g.*, *cholam* malt diastase, it is found that a definite and large fraction of the total reducing power (saccharifying capacity) is associated with the liquefying component, and only a smaller fraction conforms to the true " saccharogenase " of Ohlsson.

(7) It, therefore, seems sound to conclude that diastase consists not of a dextrinogenase and a saccharogenase according to the hypothesis of Ohlsson, but of two components which we can conveniently denominate as α-amylase and β-amylase, each possessed of a definite amount of saccharifying power, as measured by the total reducing power produced. α-amylase causes the liquefying power, as noted by the iodine colour change, and presumably acts on the amylopectin fraction of starch, producing a series of dextrins, the higher members of which (erythro-dextrins) give the characteristic colouration with iodine; the rapid fall of viscosity of starch solution immediately after the addition of diastase, is also associated with the amylopectin fraction and α-amylase.

β-amylase corresponds to the true "saccharogenase" of Ohlsson and presumably acts on the "amylose" fraction of starch, decomposing it into maltose and dextrins not giving a colour with iodine. Now, more work is necessary before some of the ideas underlying the above hypothesis, can be substantiated, but the present hypothesis would appear to explain the observed facts in regard to *cholam* and barley malt diastases, better than the original hypothesis of Ohlsson.

According to the present hypothesis, barley malt diastase contains a large proportion of β-amylase (33 mgrms. out of 36 mgrms.), and only a small amount of α-amylase, while *cholam* malt diastase contains a large proportion of α-amylase (27 out of 36 mgrms.), and only a small quantity of β-amylase.

(8) Now, the liquefying power (α-amylase fraction) of two diastases can be compared either (1) by comparing the reciprocals of times required in the two cases for the change of iodine colour to purple, or (2) by directly comparing the reducing powers associated with the α-amylase fractions in the two diastases. As both methods should give the same result, the ratio of reducing power associated with α-amylase to reciprocal of time required for change of iodine colouration to purple should be constant for α-amylases obtained from different diastases, when examined under similar conditions of substrate, temperature, pH, etc. Table V shows that this ratio is about 11 in the case of both *cholam* and barley malt diastases; this would appear to a striking confirmation of the soundness of the present theory of α- and β-amylases.

A further study of the action of α- and β-amylases was therefore made in order to see whether they can satisfactorily explain the differences between the properties of *cholam* and barley malt diastases noted at the beginning of this paper.

Experiment (6). Identity of the β-amylases from barley and cholam *malt diastases in regard to the kinetics of their action on starch.*

In order to obtain β-amylase preparations of equal activity from *cholam* and barley diastases, 0·05 grm. of barley malt diastase and 0·10 grm. of *cholam* malt

diastase were separately dissolved in 10 c.c. of water and filtered. Four c.c. of the filtrate were taken in a test-tube and treated according to the procedure given in Expt. (1), but keeping the tubes in the ice-chest for 2 hours, instead of 15 minutes. The following values were obtained :—

TABLE VI.

Time	Barley			*Cholam*		
	R. P. of 10 c.c. in mgrm. Cu	*R*	Iodine colour	R. P. of 10 c.c. in mgrm. Cu	*R*	Iodine colour
1 hour . . .	20	10·0	Blue .	19	9·5	Blue
2 „ . . .	38	19·0	„ .	37	18·5	„
4 „ . . .	73	35·0	„ .	70	35·5	„
6 „ . . .	92	46·0	„ .	93	46·5	„
8 „ . . .	105	52·5	„ .	107	53·5	„
10 „ . . .	112	56·0	Purple .	115	57·5	Purple
24 „ . . .	121	60·5	Violet .	127	63·5	Red
48 „ . . .	132	66·0	Red . .	142	71·0	Brown

The above figures show that the curves for β-amylase are alike, both in regard to change in iodine colouration and changes in reducing power, showing that they are decomposing starch in a similar manner, even though they are obtained from such widely different diastases like barley and *cholam*.

Experiment (7). *Identity of the α-amylases from barley and* cholam *diastase in regard to the kinetics of their action on starch.*

In order to obtain α-amylases of equal activity from barley and *cholam* malt diastases, 0·1 grm. of the former and 0·01 grm. of the latter were disolved in 10 c.c. of water and filtered. The filtrate was kept at 62°-63°C. for 15 minutes and 5 c.c. of the treated enzyme solution added to 200 c.c. of 2 per cent. starch solution, buffered to pH 5·0 and kept at 30°C. Ten c.c. aliquots were taken for analysis.

The figures obtained which are given in Table VII, show that the α-amylase curves for *cholam* and barley are in essential agreement with one another, both in regard to the stage at which the iodine-colour change is produced and in regard to the general shape of the curves. In both these respects, the curves for α-amylase widely differ from those of β-amylase obtained in Expt. (6), (Fig. 13).

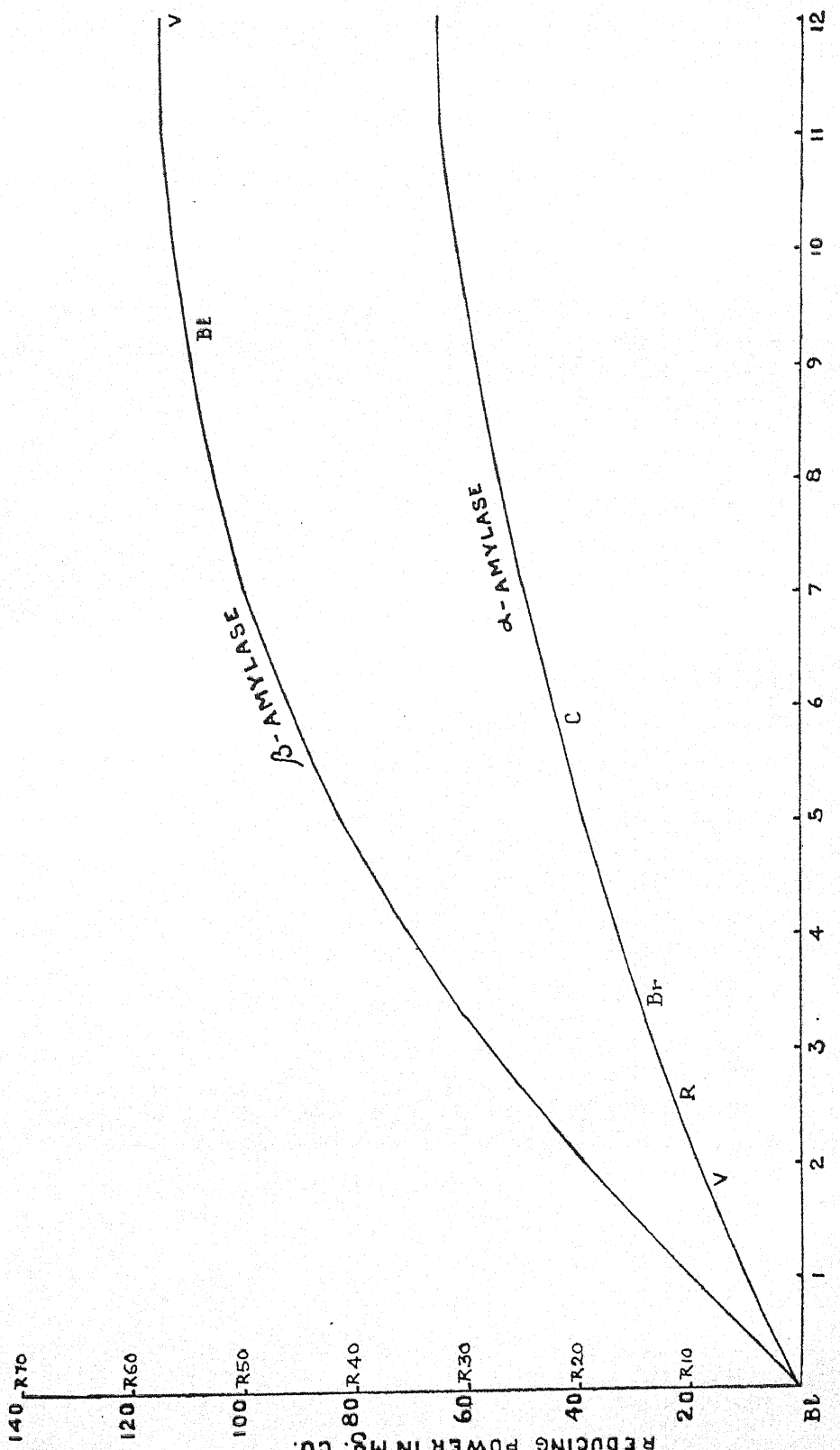


Fig. 13. Saccharification by α- and β-amylases.

TABLE VII.

Time	Barley			*Cholam*		
	R. P. of 10 c.c. in mgrm. Cu	*R*	Iodine colour	R. P. of 10 c.c. in mgrm. Cu	*R*	Iodine colour
. . .	10	5·0	Blue . .	12	6·0	Blue
. . .	15	7·5	Purple .	17	8·5	Violet
. . .	18	9·0	Violet .	22	11·0	Red
. . .	25	12·5	Red . .	30	15·0	Brown
. . .	32	16·0	Brown .	38	19·0	Light brown
. . .	44	22·0	Light brown	50	25·0	Colourless
. . .	50	25·0	Colourless .	55	27·5	,,
. . .	80	40·0	,,	84	42·0	,,
. . .	99	49·5	,,	103	51·5	,,
. . .	110	55·0	,,	115	57·5	,,
. . .	116	58·0	,,	125	62·5	,,

Experiment (8). Production of glucose by the action of α-amylase on starch.

; has already been shown that when *cholam* or barley diastase is heated ution to 60°C. or higher, glucose could be identified among the products drolysis. Succeeding work reported above on α- and β-amylases made it ble that the formation of glucose may be due to the action of α-amylase on . In order to test this point, experiments were repeated on the lines of the . (6) and (7), and the α- and β-amylases obtained from *cholam* and barley ses were reacted with starch. Toluene was added as preservative.

liquots of 20 c.c. of the starch hydrolysate were taken at intervals and sed for the amount of glucose present by the glucosazone method. Glucose not be detected among the products of hydrolysis of the β-amylases *cholam* and barley malt diastases. But in the case of the α-amylase glucose be identified in the form of its osazone, in about a week in the case of α-amylase and about 10 days in the case of barley α-amylase. It must ted that the enzymes were taken in comparatively weak concentrations, of the two, the *cholam* α-amylase was in stronger concentration. A larger

quantity of glucosazone, representing about 10 per cent. conversion of the starch into glucose was finally obtained at the end of a fortnight in the case of *cholam* α-amylase, while a yield of about 5 per cent. was obtained in the case of barley α-amylase. This difference might be due to the fact that the barley enzyme taken was weaker.

Ling and Nanji [1925] have suggested that glucose is formed from the amylopectin fraction of starch by the action of diastase at high temperatures. The present experiments are in agreement with this observation, and furnish additional evidence to show that the glucose is formed by the action of α-amylase on starch, presumably on the amylopectin fraction. At ordinary temperatures, even though glucose may be produced similarly by the action of α-amylase, the detection of it as the osazone is rendered impossible on account of the large excess of maltose, whose presence effectively suppresses the precipitation of glucose as osazone.

Discussion.

A comparison of the α- and β- amylase curves shows marked differences, both in the velocity of action and in the manner of decomposition of starch (Fig. 13).

The β-amylase curve maintains its initial speed for a longer time and resembles the barley curve. The iodine-purple change is shown by β-amylase at about 60 per cent. "apparent maltose", *R* 60, while in the case of barley, it is shown at about 50 per cent. apparent maltose, *R* 50. This resemblance between the two curves is natural, since it has been shown that barley malt diastase contains a large proportion of β-amylase.

The α-amylase curve, on the other hand, resembles the curve of *cholam* diastase, in that it shows a falling off in the rate of saccharification from the early stages. The iodine colour change comes at a very early stage. In the case of α-amylase, the purple colour is reached at about 7½ per cent. of apparent maltose (*R* 7·5), whereas in the case of *cholam*, the purple colour appears at about 10 per cent. apparent maltose (*R* 10). This resemblance is corollary to the fact that *cholam* malt diastase contains a large proportion of α-amylase.

It is evident from the nature of the iodine colour changes in the two cases that α- and β- amylases act on different portions of starch, the α-amylase on the amylopectin fraction and the β-amylase on the amylose fraction.

The separation of diastase into two such constituents like α- and β-amylases having widely different properties satisfactorily explains the differences in the properties of diastases from different sources and specially the marked differences between the action of *cholam* and barley malt diastases on starch, noted in the beginning of this paper.

Firstly, it explains the greater liquefying but lower saccharifying power of *cholam* diastase, as compared with barley, on the basis that *cholam* diastase contains a larger amount of α-amylase (liquefier) and a smaller amount of β-amylase (saccharifier), as compared with barley malt diastase.

Secondly, it helps us to understand the characteristic differences existing in the saccharification curves of *cholam* and barley, as being due to the large percentages of α- and β-amylases respectively, contained in the two diastases.

Thirdly, it explains why *cholam* produces at the earlier stages more dextrin and less maltose (due to α-amylase acting on amylopectin), while barley diastase produces more maltose and less dextrin (due to β-amylase acting on " amylose ").

Fourthly, it explains why the curves for *cholam* are similar both at ordinary and at high temperatures, while in the case of barley, they are widely different. *Cholam* diastase contains largely α-amylase, which is stable at high temperatures (60°C.), and hence the curves are the same both at ordinary and at high temperatures. Barley malt diastase, at ordinary temperatures, shows the characteristic curve of β-amylase, of which it contains a large proportion, but when the enzyme is heated to above 60°C. the β-amylase fraction is destroyed, and hence the resulting curve follows that of the α-amylase fraction, which is widely different from the previous curve and resembles that of *cholam*. The curves for *cholam*, therefore, both at ordinary and at high temperatures, resemble those of barley at high temperatures.

Fifthly, it explains the formation of glucose by diastatic action, when the diastase has been previously heated to above 60°C., under which conditions only α-amylase is present which produces glucose from starch. Further experimental evidence is thus offered in support of Ling and Nanji's observation that glucose is produced from the amylopectin fraction of starch.

Lastly, the presence of the two amylases in diastase may satisfactorily account for the two different pH optima observed for *cholam* diastase, at pH 4·8 and 6·0. pH 4·8 probably represents the optimum for β-amylase, while pH 6·0 represents the optimum for α-amylase, but more work is necessary to confirm this.

Summary.

(1) Experimental evidence is offered which goes to show that diastase consists of two components—α-amylase and β-amylase.

(2) α-amylase is the liquefying agent and possesses a definite amount of saccharifying power (as measured by total reducing power). It presumably acts on the amylopectin fraction of starch, decomposing it into erythro-dextrins (which colour iodine), achroo-dextrins and maltose. It is stable at 60°C., and can be

separated from β-amylase by heating the diastase in solution at 62°-63°C. for 10-15 minutes.

(3) β-amylase is stable at 0°C. and pH 3·2 and can be isolated from α-amylase by keeping the diastase in solution at 0°C. and pH 3·2 for about an hour. It acts on the amylose fraction of starch, producing maltose and dextrins.

(4) It is shown that *cholam* malt diastase contains a large proportion of α-amylase and only a small proportion of β-amylase, while the reverse is the case with barley malt diastase.

(5) The marked differences noted between *cholam* and barley malt diastases, in their action on starch, can be satisfactorily explained on the basis of the difference in the relative amounts of α- and β-amylases contained in them.

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INHERITANCE OF ALBINO AND WHITE-STRIPED CHARACTERS IN RICE.

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(With Plates XXXV and XXXVI.)

Rice seedlings deficient in chlorophyll are noticed every year scattered here and there in the seed-bed of apparently pure types. Most of them are entirely chlorotic (albino) which soon die in the seed-bed, while a few are partially so giving a variegated appearance to the plants with white-striped leaves. The latter survive although in a weaker condition than the green plants, and produce normal grains. These chlorophyll abnormalities are on the whole very few in comparison to the green population and especially the white-striped plants which are very rare.

Previous work.

Working with maize Brunson [1924] observed that the pale green plants which died in the seedling stage showed the character to be a simple Mendelian recessive.

Kondo [1928] observed the white and green variegated plants occurring spontaneously in several lines of rice giving out albino, variegated and green plants in different percentages. He further observed that variegation was not transmitted through the pollen. Variegated plants gave the same type of offspring whether selfed or pollinated with the pollen from the green plants. He concluded that the character was non-Mendelian maternal in inheritance.

Ramiah [1930] also studied the lethal albino and pale yellow seedlings in rice. The former died a few days after sowing while the latter attained maturity like the normal greens although the rate of growth was slower. Moreover, the pale yellow character was distinguishable only in the early stage but disappeared with growth. The occurrence of both albino and pale yellow seedlings was more common in the progenies of crosses and they were noted to be recessive in character segregating in the ratios of 3 : 1 and 15 : 1 of green to white or yellow.

(*a*) *Albino character.*—Albino seedlings have been found to appear in a number of pure strains in rice as well as in crosses every year in the rice experiment stations at Karimganj and Titabar. They may grow for about ten or fifteen days, when they die in the seed-bed for lack of chlorophyll in them, *i.e.*, as soon as they have

exhausted the food material in the endosperm. Attempts were made to treat them with ferric chloride in well manured earthen pots, but they never improved, evidently showing that they have not the capacity to grow in the normal way. The grains that possess this albino character cannot be distinguished at the beginning of germination but as the caulicle remains colourless they are easily recognised among the green seedlings after five or six days. The following table will show the percentage of albino seedlings* noted in a number of pure strains of rice and a few progenies of crosses at the Karimganj Farm in 1929 :—

TABLE I.

Showing percentage of albino seedlings in rice.

Variety	Total No. of plants	Green	Albino	Percentage
A. *Aus.*				
M. $\frac{36}{17}$	117	116	1	·85
" $\frac{36}{30}$	256	249	7	2·73
" $\frac{39}{1}$	131	129	2	1·52
" 156	285	281	4	1·40
As. 2	290	286	4	1·40
" $\frac{36}{2}$	166	148	18	10·80
47	322	317	5	1·55
F_7—As. C. $\frac{6}{129}$ b	328	325	3	·91
" $\frac{6}{130}$	357	351	6	1·70
" $\frac{6}{131}$ a	211	208	3	1·42
F_3—As. C. $\frac{363}{3}$	93	80	13	13·98
" $\frac{363}{40}$	64	52	12	18·75
" $\frac{363}{49}$	109	90	19	17·40

*A few yellowish white seedings were also noted with the albinos, but as they did not survive they were counted as albinos.

TABLE I—*contd.*

Variety	Total No. of plants	Green	Albino	Percentage
A—*contd*				
F_3—As. C. $\frac{363}{50}$	128	119	9	7·03
„ $\frac{363}{61}$	108	101	7	6·50
„ $\frac{363}{66}$	76	61	15	19·74
B. *Sail*				
S. 20	234	233	1	·43
„ 27	218	214	4	1·83
„ 33	131	130	1	·76
„ 48	218	217	1	·46
„ $\frac{182}{1}$	164	163	1	·61
„ 187	167	163	4	2·40
„ $\frac{241}{5}$	216	215	1	·46
„ $\frac{241}{9}$	152	148	4	2·63
„ 268	151	150	1	·66
„ $\frac{383}{2}$	116	114	2	1·72
„ 475	322	321	1	·31
„ 484	263	262	1	·38
„ 535	338	337	1	·30
„ 537	385	382	3	·78
C. *Asra*				
Ar. 39	220	219	1	·45
„ $\frac{67}{4}$	388	387	1	·26
„ 78	781	770	11	1·40
„ 79	500	497	3	·60
„ 110	351	350	1	·28
„ 2	313	312	1	·32

The above table shows that the percentage of albino seedlings ranges from ·26 per cent. to 20 per cent. without showing any definite ratio and the crosses show a larger percentage than the ordinary pure strains of rice as also pointed out by Ramiah [1930]. Moreover, the percentage of albino seedlings given out by the same variety year after year varies a great deal while the majority of the varieties do not show them at all. The frequent appearance of such lethals in different strains of rice seems to indicate the occurrence of new mutations in nature as has been pointed out by Brunsen [1924] in maize and Rangaswami Ayyangar in millet [1932].

(*b*) *White-striped character.*—In 1926 three plants with white-striped leaves appeared at the Titabar Farm in one of the apparently pure seed-bed of S. 559, Sualkuchi, collected from the district of Goalpara in Assam. They were isolated and transplanted separately. The plants were harvested and numbered as Nos. 1, 2 and 3. As they were identical in their leaf and grain characters only No. 3 was continued in successive generations for experimental purposes.

In the next generation some of the plants of the parent variety Sualkuchi, produced only green seedlings while others produced green, white-striped and albino seedlings. Moreover, the grains of the white-striped plant No. 3 produced in the next generation whited-striped and albino seedlings together with a few scattered greens Plate XXXV. The following table will show the segregation of the original parent plant Sualkuchi and the white-striped plant No. 3.

TABLE II.

Showing the segregation of the original green parent, Sualkuchi and the white-striped plant No. 3.

Variety	No. of observations	Number of			Percentage of		
		Green	White-striped	Albino	Green	White-striped	Albino
a. Sualkuchi (green homozygous-**GGWW**or **GGww**).	536	535	..	1	100	..	..
	308	308	..	..	100	..	..
b. Sualkuchi (green heterozygous-**GgWw**).	639	444	142	53	69·48	22·22	8·30
	116	82	26	8	70·69	22·41	6·90
	132	84	38	10	63·64	28·79	7·57
	Average				68·77	23·22	8·01
c. No. 3 (White-striped-**ggWw**)	345	5	226	114	1·45	65·51	33·04
	70	1	47	22	1·43	67·14	31·43
	58	2	39	17	3·45	67·24	29·31
	287	5	226	56	1·75	78.74	19·51
	82	..	65	17	—	79·27	20·73
	Average				1·55	71·61	26·84

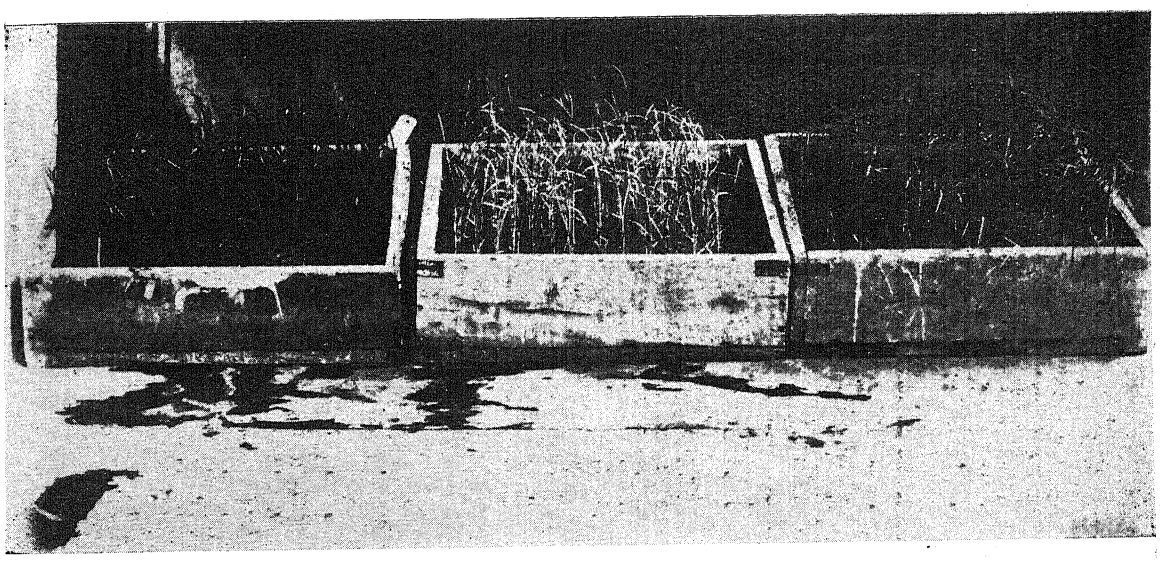


Fig. 1. Showing homozygous green seedlings (left), heterozygous green seedlings with scattered albinos (right), and white-striped seedlings with albinos and a few scattered greens (middle).

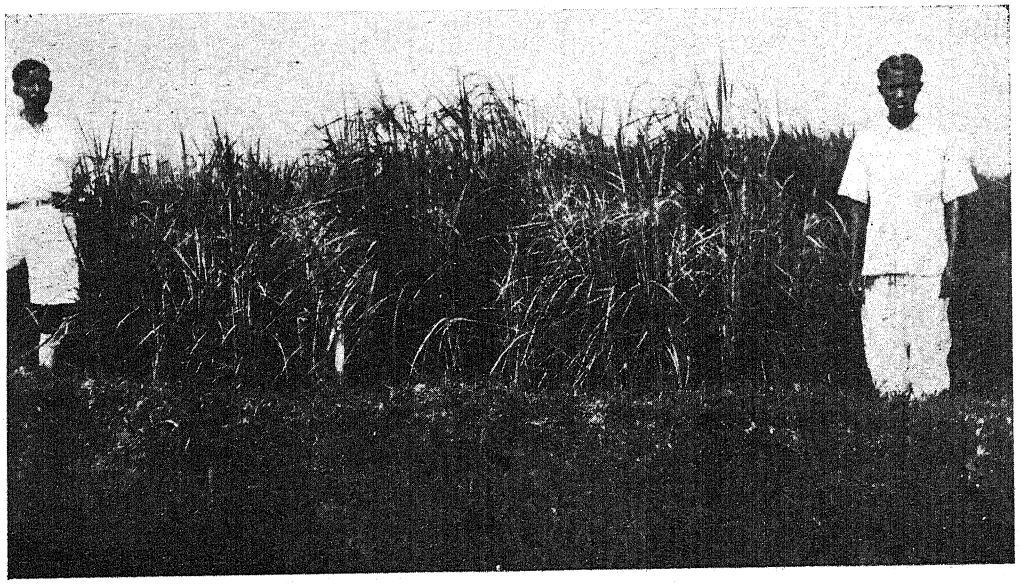


Fig. 2. Showing the size of the white-striped and green plants in the same plot.

The above table shows that the parent plant, Sualkuchi, produced 100 per cent. green seedlings in (*a*), whereas in (*b*) it produced on average 69 per cent. green, 23 per cent. white-striped and 8 per cent. albino seedlings in the ratio of 12 : 3 : 1 approximately. In (*c*) the white-striped plant No. 3 produced 71·6 per cent. white-striped, 26·8 per cent. albino and 1·6 per cent. green seedlings, the last of which was suspected to be the results of natural crosses as confirmed later on by the ratio (3 : 1) obtained from six natural crosses in Table IV. If the green plants are left out the remaining seedlings give a ratio of 3 white-striped : 1 albino, showing fairly a good fit.

From the above results it can be supposed that a natural crossing occurred between the original parent Sualkuchi (**GGww**) and a white-striped mutant (**ggWW**) originating in the same plot. Accidentally perhaps the crossed grain was selected and grown, of which, some of the F_2 plants might be supposed to have been dealt with in Table II. The genetic constitution of the three phenotypes may, therefore, be analysed as below :—

Parents	**GGww** (Green Sualkuchi)		
	ggWW (White-striped mutant)		
F_1	**GgWw** (heterozygous green)		
F_2	1 **GGWW**	1 **ggWW**	1 **ggww**
	2 **GGWw**	2 **ggWw**	
	2 **GgWW**		
	2 **Ggww**		
	4 **GgWw**		
	1 **GGww**		
	12 Green :	3 White-striped :	1 albino.

Here the factor **G** stands for green and **W** for the white-striped character and **g** and **w** their respective allelomorphic absences. In the presence of **G** the plant will be green. In the absence of **G** and presence of **W** the plant will be white-striped and in the absence of both the plant will be albino which is a lethal character. Here the cause of the lethal effect produced by two albino factors acting together is evident from the fact that they prevent the development of chlorophyll essential in the metabolism of the plants and they die within a few days from germination.

Recessive lethals, however, in most species tend to be eliminated in time in competition with their normal allelomorphs. The number of lethal albinos, therefore, becomes less in each succeeding generations and gradually approaches ex-

tinction. We thus got an almost pure white-striped population from the plant No. 3 in subsequent generations.

The vegetative characters of pure green, Sualkuchi and the white-striped plant No. 3 differ a good deal. The white-striped plant looks rather sickly. Even the grains, when immature, show distinctly whitish ribs. The inner glume attain a very light yellow colour on maturity. Actual measurements have shown that the white-striped plant is a degenerated type when compared with its original parent plant as shown in the table below :—

TABLE III.

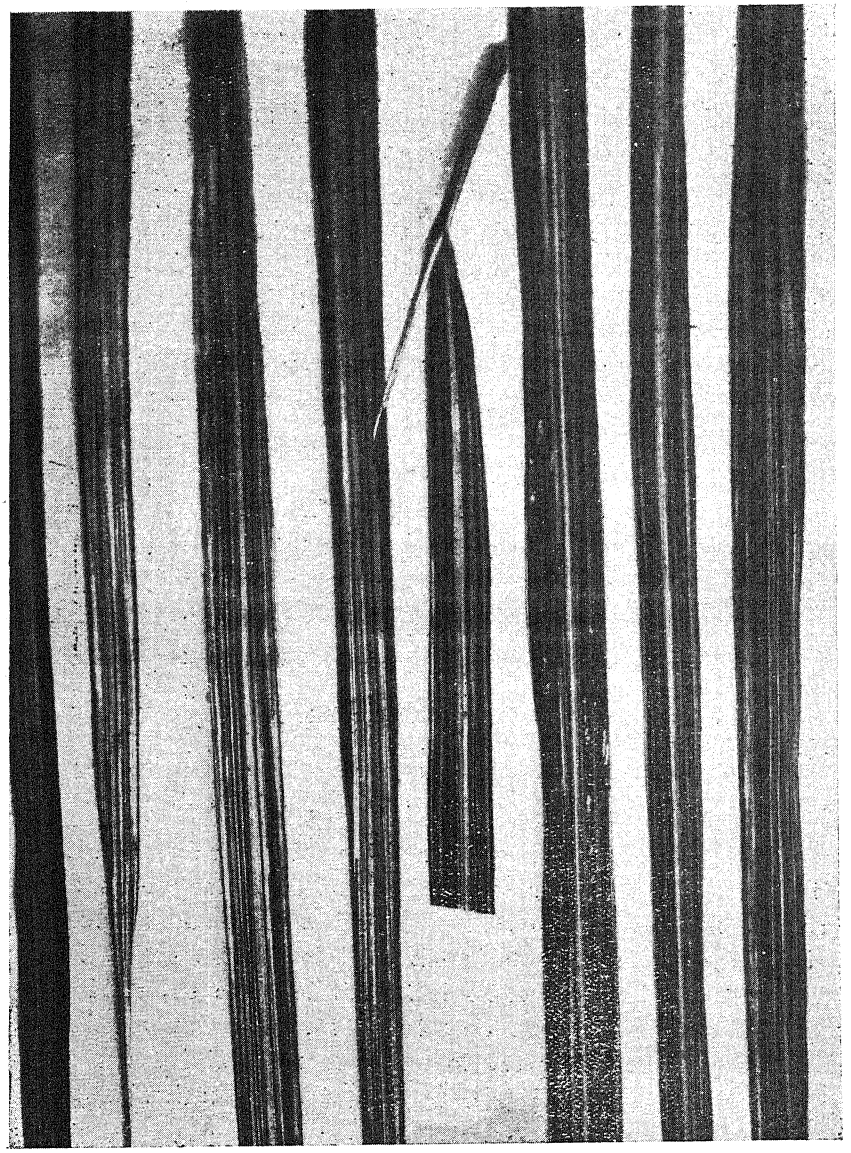
Showing the main vegetative characters of pure green Sualkuchi and white-striped plant No. 3.

Variety	Length of straw in inches	No. of tillers per plant	Colour of inner glume	Yield in tolas per 100 plants
Sualkuchi (green)	48	9·4	Deep yellow with deep brown furrows	141·125
No. 3 white-striped	25	5·4	Light yellow with light brown furrows	53·75

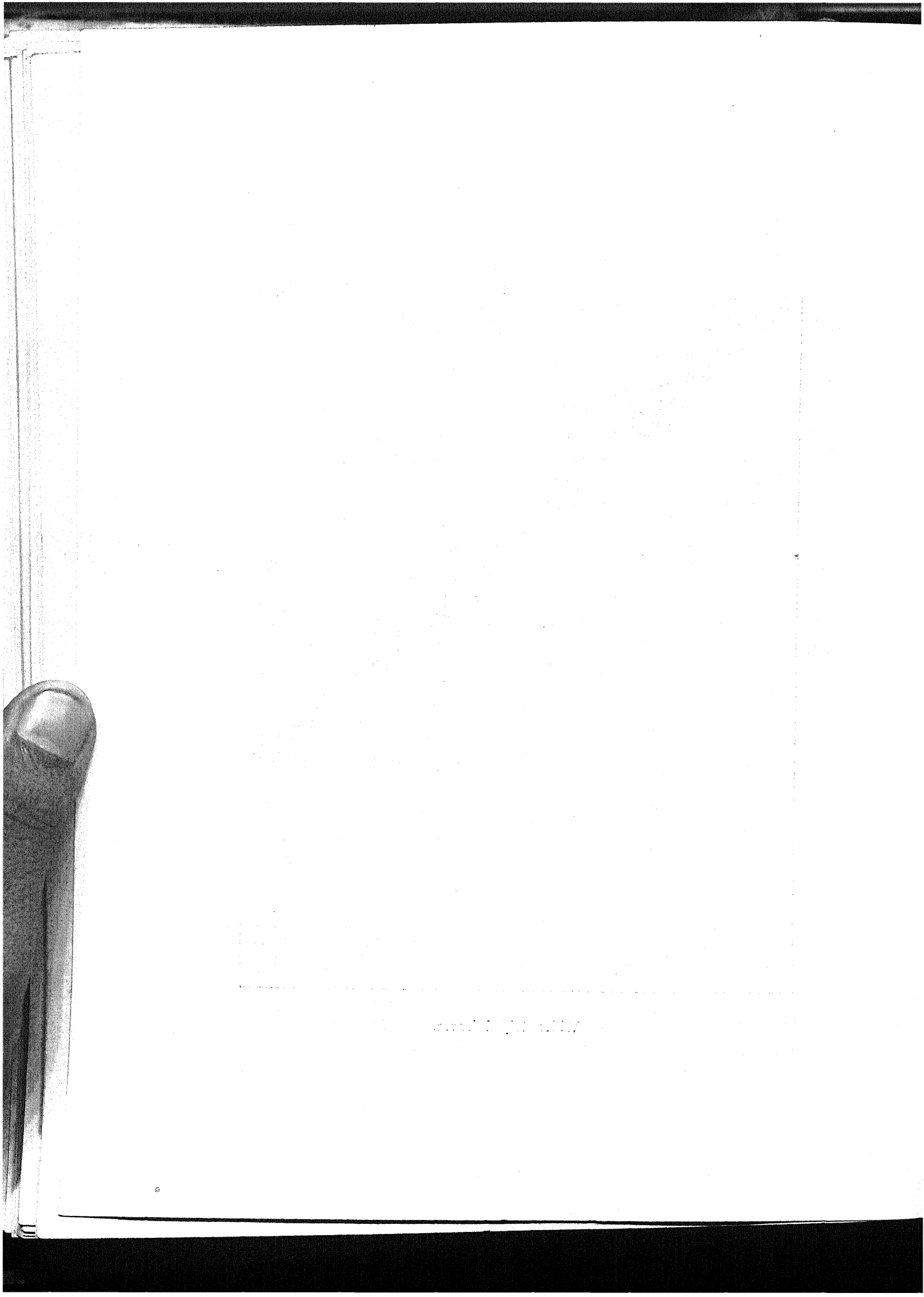
Ramiah [1930] has noted that the difference observed in the early stages of the pale yellow seedlings disappears later on, for the leaves turn green, but in our case the white-striped character can easily be distinguished up to maturity and the mature seeds are also distinguishable by their lighter colour from the seeds of the normal green plants.* Attempts were then made to study the inheritance of the white-striped character in rice both from natural and artificial crosses.

Natural cross.—Some seeds of the white-striped plant No. 3, were grown at the Karimganj Farm in 1929. When sown in the seed-bed some of the seedlings proved to be entirely chlorotic which withered away a few days after. Of the remaining plants six were found to be entirely green and the rest of the 200 plants were white-striped. The white-striped plants bred true in the following generations but the six green plants which were suspected to be crossed naturally with some green variety were all grown separately and selfed. In the F_2 all of them

* Recently, it has come to the notice of the writers that some young seedlings with white-striped leaves at the beginning changed entirely to green plants later on. This character is under investigation.



White-striped leaves.



segregated approximately in the ratio of 3 green : 1 white-striped as shown in the table below :—

TABLE IV.

Showing the F_2 generation of the natural crosses.

Plant No.	Observed		Calculated 3 : 1		Total	Deviation	D./P. E.
	Green	White-striped	Green	White-striped			
1	115	52	125·25	41·75	167	10·25 — 3·78	2·71
2	171	50	165·75	55·25	221	5·25 — 4·34	1·21
3	177	53	172·5	57·5	230	4·5 — 4·43	1·02
4	297	75	279·0	93·0	372	18·0 — 5·63	3·20
5	265	79	258·0	86·0	344	7·0 — 5·41	1·29
6	139	41	135·0	45·0	180	4·0 — 3·92	1·02
Total .	1,164	350	1,135·5	378·5	1,514	28·5 — 16.85	1·69

Except the Nos. 1 and 4 the rest of the plants show a good fit to the calculated numbers. The plant No. 1 was continued in F_3 and the results confirmed the above ratio as follows :—

TABLE V.

Showing the F_3 results of the natural cross plant No. 1.

F_2	F_3		
	Segregations	Observed	Expected
115 Green	Pure	41	38
	Segregating (3 : 1) . . .	74	77
52 White-striped	Pure	51	52
	Segregating	1	*nil*

The one white-striped plant which segregated in F_3 is suspected to be a case of natural cross.

It may well be noted here that the genotypic constitution of the green and white-striped parent plants in these natural crosses may be supposed to be **GGWW** and **ggWW** respectively which will not throw any albinos in F_2. The albinos should only come out from the heterozygous white-striped plants as in Table II having the genotypic constitution of **ggWw**.

The heterozygous green plant No. 1 (**GgWW**) was backcrossed with the recessive white-striped character (**ggWW**) and out of 31 successful grains 17 proved to be green and 14 white-striped with a deviation of 1·5—1·87 from the expected number 15·5 in each case.

Artificial cross.—To confirm the results of natural crosses a few artificial crosses were also tried between the white-striped and green plants. The F_1 plant was green and the F_2 plants segregated approximately in the ratio of 3 green : 1 white-striped as shown in the table below :—

TABLE VI.

Showing the F_2 generation of the artificial crosses.

Cross No.	Parentage	Green	White-striped	Total
700	S. 22 (green)—**GGWW** × white-striped **ggWW**	90	36	126
708	White-striped **ggWW** × S. 22 (green)—**GGWW**	86	34	120
	Observed total	176	70	246
	Calculated 3 : 1	184·5	61·5	246

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{8{\cdot}5}{6{\cdot}79} = 1{\cdot}25.$$ The fit is good.

The cross No. 700 was continued in F_3, but the results only confirmed the above ratio. A few albino seedlings were again noticed in the seed-bed, which did not, however, exceed 1·5 per cent. on average. This agrees with the occurrence of albinos in pure types, as shown in Table I. The albinos here have already been stated as natural mutation. The difference between the white-striped plant in this case as

well as the white-striped plant No. 3 in Table II lies in the fact that the genotypic constitution of the former is **ggWW** and of the latter **ggWw**.

The following table will show the results of growing the F_2 individuals of cross No. 700 in F_3 as follows :—

TABLE VII.

Showing the F_3 results of the artificial cross.

F_2	F_3		
	Segregations	Observed	Expected
90 green	Pure	27	30
	Segregating (3 : 1)	63	60
36 white-striped	Pure	36	36

The results of both natural and artificial crosses show that the inheritance is Mendelian and that not more than two pairs of factors are involved in the crosses studied.

Kondo [1928] observed that variegation was not transmitted through the pollen and variegated plants gave the same type of offspring whether selfed or pollinated. But in our case the white-striped character was always recessive to green and behaved as a true Mendelian character giving the same kind of offspring whether used as male or female.

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DETERMINATION OF NITROGEN IN SOILS, IV.

PRE-TREATMENT WITH OXIDIZING AGENTS AND ITS INFLUENCE ON THE PROGRESS OF ACID DIGESTION.

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The use of oxidizing agents to promote the digestion of organic materials is well known. Treatment with hydrogen peroxide to hasten the micro-Kjeldahl digestion of organic substances would appear to have been in vogue in Victor Meyer's laboratory though there is no mention of it in any of his publications. In his micro-method for the estimation of nitrogen in biological fluids, Rose [1925] recommends the use of small quantities of hydrogen peroxide as a catalyst. Christensen and Fulmer [1927] and later, Thorne [1932] refer to increase in the estimate of total nitrogen in yeast as the result of pre-treatment with hydrogen peroxide. Among the other oxidizing agents, mention may be made of perchloric acid, the use of which is recommended by Mears and Hussey [1921]. Doneen [1932] also reports rapid micro-digestion of plant materials in presence of the same acid.

Evidence has already been adduced to show that pre-treatment with hydrogen peroxide leads to increased efficiency in the acid digestion of soils [Sreenivasan and Subrahmanyan, 1933]. It was also noted that the progress of digestion was then so rapid that, in some cases, whereas digestion by the official method was incomplete even at the end of six hours, similar specimens treated with hydrogen peroxide were fully digested in half-an-hour. In view of the practical significance of the foregoing observations, further work was undertaken with a view to (*a*) determining the nature and extent of influence of hydrogen peroxide and other oxidizing agents on the reaction between soil and sulphuric acid and (*b*) standardising the conditions for an improved method of acid digestion of soils.

Pre-treatment with barium peroxide.—In view of the high cost of hydrogen peroxide and the fact that the commoner preparations of that reagent contain nitrogen [Sreenivasan and Subrahmanyan, *loc. cit.*], some experiments were carried out to determine whether barium peroxide can be used in its place. The soil (5 grms.) was first treated with 40 c.c. of 1 : 1 sulphuric acid and then with varying quantities of the peroxide and hydrated sodium sulphate. The digestions were all

stopped at the end of one hr. and the results compared with those obtained after 'wet' digestion for one and 1½ hrs. respectively (Table I).

TABLE I.

Treatment		Nitrogen in parts per million (averages)			Standard Error
BaO_2 in grms.	$Na_2SO_4 10H_2O$ in grms.	Soil-Red clay —Surface— NANDYAL	Soil-Light black cotton —Surface— SHOLAPUR	Soil-Uplands —Surface— GORAKPUR	
2	6	286·2	310·7	472·5	±2·1
3	4	299·5	335·3	499·1	±1·6
4	2	297·3	332·8	496·9	±1·2
5	0	299·5	339·5	496·9	±1·1
'wet'—1 hour		281·8	299·5	461·5	±2·3
'wet'—1½ hours		301·7	337·2	496·9	±1·2

As may be seen from the results, the digestion of all the specimens receiving more than 3 grms. of the peroxide was complete at the end of one hr. The addition of sodium sulphate seems to be unnecessary since as good results were obtained without it as with it.

Experiments with sodium peroxide.—In view of the precipitation of barium sulphate on addition of alkali and the consequent difficulty in distillation, a 10 per cent. solution of sodium peroxide in ice-cold, 1:1 sulphuric acid was tried as the digestion mixture. The results (Table II) show that the digestion was complete in one hour.

TABLE II.

Treatment	Nitrogen in parts per million (averages)			Standard Error
	Soil-Red clay —Surface— NANDYAL	Soil-Lowland Surface-paddy— CUTTACK	Soil-black cotton-surface— INDORE	
'Dry'—(Official) 2 hours.	244·0	754·3	395·0	±2·4
'Wet'—1 hour . .	281·8	732·1	516·9	±1·9
,, —1½ hours . .	301·7	767·6	532·5	±0·8
Na_2O_2—1 hour . .	303·9	765·4	530·2	±0·7

The acid solution of sodium peroxide is not quite stable so that only small quantities of that reagent should be prepared at a time. Care should also be exercised in the preparation, as the reaction between sodium peroxide and acid is rather violent and might lead to considerable loss of hydrogen peroxide if the temperature is not maintained low.

Rates of digestion by different methods.—Specimens (5 grms.) of two soils, one from Nandyal and the other from Nasik, were digested by different methods and the progress of digestion determined at half hour intervals (Table III). The heating was carried out over electric heaters which had been previously standardised for the work.

TABLE III.

Mode of digestion	Nitrogen as parts per million (averages)					Standard Error
	Time of digestion in minutes					
	30	60	90	120	150	
	SURFACE SOIL FROM NASIK—DRYLAND					
'Dry' (Official)	763·2	816·4	842·9	860·8	856·3	±2·9
'Wet' (Overnight) . . .	878·4	89[illegible]·7	909·5	905·1	909·5	±1·4
Pre-treatment with H_2O_2 (6 per cent.).	905·1	909·5	909·5	905·1	..	±1·5
	SURFACE SOIL FROM NANDYAL—RED CLAY					
'Dry' (Official) . . .	226·2	235·2	244·0	252·8	248·5	±3·1
'Wet' (Overnight) . . .	275·1	297·3	306·1	301·7	301·7	±1·7
Pre-treatment with H_2O_2 (6 per cent.)	301·7	306·1	301·7	301·7	..	±1·4

It may be seen from the above that the digestion of the specimen treated with hydrogen peroxide was complete in half-an-hour while that of the 'dry' treated one was incomplete even at the end of $2\frac{1}{2}$ hours. The rate of digestion of the 'wet' treated specimens was intermediary between both.

Standardisation of conditions for addition of barium peroxide.—Into one set of flasks barium peroxide was weighed out in lots of 0·5, 1·0 and 1·5 grms. respectively and 40 c.c. of 1:1 sulphuric acid added to each. To another set, the acid was first

added and then peroxide introduced in small quantities at a time until the same total quantity as in the previous set had been added. After the reaction was completed, the resulting hydrogen peroxide was estimated by titration against standard permanganate. The percentage efficiency in the conversion of barium into hydrogen peroxide was calculated in each case (Table IV.)

TABLE IV.

BaO_2 in grms.	Percentage conversion to hydrogen peroxide		Standard Error
	Acid to oxide	Oxide to acid	
0·5	83·5	89·4	±3·1
1·0	64·1	74·1	±2·7
1·5	55·3	64·7	±1·9

Addition of the oxide to the acid, would appear to be more satisfactory than the reverse, but even then the loss is so considerable, particularly with increasing quantities, that further improvement in the technique is needed to secure the maximum efficiency out of the treatment.

Substitution of hydrochloric acid for sulphuric acid.—In the previous experiment it was observed that barium sulphate, formed as the result of the reaction, tended to deposit on the unattacked peroxide and thus interfered with the progress of further action. The experiment was therefore repeated using normal hydrochloric acid in place of sulphuric acid (Table V).

TABLE V.

BaO_2 in grms.	Percentage conversion to hydrogen peroxide		Standard Error
	Acid to oxide	Oxide to acid	
0·5	94·1	98.8	2·1
1·0	88·8	88·8	1·6
1·5	69·4	70·6	1·4

It was observed that the reaction proceeded more rapidly than in the previous experiment. The efficiency of conversion into hydrogen peroxide, though slightly

improved, was still unsatisfactory. No perceptible difference could be noticed between the two sets of treatments.

Mode of action of hydrogen peroxide.—The nature of reaction between soil and hydrogen peroxide has already been the subject of a few enquiries, particularly by Robinson and Jones [1925], Robinson [1927], Waksman and Stevens [1930], Richardson [1931], and McLean [1931], but the available evidence is not sufficient to explain how the peroxide prepares the soil for subsequent reaction with sulphuric acid. It may be expected that the preliminary oxidation by hydrogen peroxide converts the resistant forms of organic matter into readily digestible condition, but considering that a large part of the peroxide is lost by spontaneous decomposition, the actual amount concerned in the oxidation is presumably very small. In view of this and the fact that the steady evolution of oxygen keeps the soil particles well dispersed during the earlier stages of the digestion, it appeared probable that the action of the peroxide may also be mechanical, in which case any other substance producing gas by interaction with the acid should prove equally effective. With a view to throwing some light on the mode of action of the peroxide, some experiments were carried out pre-treating the soil with different substances and studying the effect thereof on the rate of digestion by the 'wet' method (Table VI.).

TABLE VI.

Treatment	Nitrogen in parts per million (averages)				Standard Error
	Time of digestion in mins.				
	30	60	90	120	
Zinc powder (1 grm.)	885·2	887·4	905·1	909·5	±2·9
BaO_2 (3 grms.)	878·4	909·5	905·1	..	±1·5
NaOBr (10 per cent.) 20 c.c. . . .	874·0	902·6	909·5	..	±1·7
BaO_2 (3 grms.)+$KMnO_4$ (0·5 grms.) .	891·7	909·5	909·5	..	±1·1
BaO_2 (3grms..)+$K_2Cr_2O_7$ (0·5 grms.) .	882·8	905·1	909·5	..	±1·4

The results show that the reaction proceeds faster in presence of different oxidising agents than in that of zinc, thereby showing that the preliminary oxidation of the soil organic matter plays a useful part in preparing the soil for subsequent digestion. Combination of the peroxide with a small amount of permanganate would appear to be a highly effective treatment.

Further observations on the relative merits of different pre-treatments.—With a view to obtaining more accurate information regarding the relative efficiencies of the different treatments, the foregoing experiment was repeated adding the different substances in quantities calculated to yield the same volumes of gases. The progress of the digestion was followed at intervals of 15 mins. (Table VII).

TABLE VII.

Pre-treatment with	Nitrogen in parts per million (averages)						Standard Error
	Time of digestion in minutes						
	15	30	45	60	75	90	
$KMnO_4$ (2·2 grms.) . . .	617·1	679·3	722·8	738·9	741·6	741·6	±2·6
Na_2CO_3 (1·1 grms.) . . .	588·5	628·4	683·3	714·5	735·0	740·3	±2·9
Zn (0·65 grms.)	580·3	633·3	688·8	725·5	747·1	745·7	±3·3
BaO_2 (5·8 grms.) . . .	638·7	728·1	729·8	743·0	741·6	...	±1·4
H_2O_2 (20 c.c. 6 per cent.) . .	684·7	743·0	744·4	741·6	..	...	±0·7
H_2O (20 c c.) (Control) . .	562·7	623·8	695·6	728·2	738·9	741·6	±1·7

It may be seen from the above that the digestion proceeds more rapidly in presence of oxidising agents than in that of the other chemicals. Among the former, it is most rapid in the case of specimens pre-treated with hydrogen peroxide, the digestion being complete in half-an-hour, while in those of others, the reaction takes nearly double that time.

It is difficult to explain why the different oxidising agents vary in their efficiency though they were all added on equivalent basis. The violence of the initial reaction that was observed in some of the cases would suggest that a considerable part of the oxygen was given off in the molecular form instead of reacting, in the nascent condition, with the soil suspension. This was particularly so in the case of the permanganate, the reaction of which with the acid was almost instantaneous. At the same time it should be mentioned that though the medium was strongly acid the reaction did not proceed to completion in the manner that may be expected from theoretical considerations ($2KMnO_4 \longrightarrow K_2O + 2\,MnO + 5O$). In presence of soil there was almost quantitative formation of manganese dioxide, thereby suggesting that the reaction proceeded as in a neutral medium ($2KMnO_4 \longrightarrow K_2O + 2MnO_2 + 3O$) so

that only three-fifths of the oxygen was immediately available for oxidation. The manganese dioxide thus formed was not readily acted on by either dilute or concentrated acid so that a large part of it remained as such even after prolonged digestion. A further difficulty in the case of the permanganate was the determination of the stage of completion, the digested mass being strongly coloured in all the cases. Similar observations would also apply to dichromates with which some preliminary experiments were carried out by the author. It would thus be seen that although oxidising agents, in general, may be useful in hastening the rates of digestion, only a few of them can prove satisfactory in practice.

The progress of the digestion in the case of specimens treated with barium peroxide would suggest that hydrogen peroxide is either not being quantitatively formed or is otherwise decomposed without reacting with the soil. Since barium peroxide is very cheap and, as distinct from commercial specimens of hydrogen peroxide, free from nitrogen, it would be desirable to modify its use in such a manner as to secure quite as satisfactory results as with pure hydrogen peroxide. Further work in this direction is in progress.

It should be admitted, however, that the use of oxidising agents precludes nitrates from the estimate of total nitrogen. The small quantities of reducing agents (*e.g.*, ferrous sulphate) used in general practice will be readily oxidised by the peroxide or permanganate which is added to hasten the digestion so that the nitrates will be left unaffected. Reaction with hot concentrated acid results in the formation of nitric acid, which being volatile or otherwise readily decomposable is lost. It is true that many soils contain only a small percentage (often less than one per cent.) of their total nitrogen in the form of nitrates, but there are others which contain quite considerable quantities in that form. It would follow, therefore, that the technique for the estimation of total nitrogen should include some treatment for reducing the nitrates to ammonia before the addition of the oxidising agent. With this end in view further work has already been taken up and will form the subject of a later communication.

Summary.

1. Pre-treatment of soil with diluted (1 : 1) sulphuric acid and barium peroxide greatly improves the rate of digestion. The resulting barium sulphate helps to raise the boiling point of the digesting acid, so that the need for adding potassium or sodium sulphate for that purpose is obviated. The use of sodium peroxide has a few advantages over that of the barium salt, but is not very safe in routine practice.

2. There is greater production of hydrogen peroxide when the barium salt is added, in small instalments at a time, to the acid than when the acid is added in one lot to the peroxide. Even then there is considerable loss of hydrogen peroxide. The use of hydrochloric acid leads to slightly better results than that of sulphuric acid.

3. The action of peroxides is essentially one of oxidation, preparing the soil for the subsequent action of acid. Mechanical agitation by different gases does not appreciably improve the rate of digestion.

4. Pre-treatment with oxidising agents, in general, helps to improve the rate of digestion, but peroxides, particularly hydrogen peroxide, would appear to be most satisfactory in practice.

5. Further work is in progress to develop a system of treatment, which would include nitrates in the estimate of total nitrogen.

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MOTES IN COTTON.

I. PUNJAB-AMERICAN COTTON.

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INTRODUCTORY.

Mature locks of cotton often contain aborted ovules which are usually called "motes". These motes not only fail to produce any fibre of commercial value, but their presence in large numbers actually lowers the price of the cotton. Motes are a cause of annoyance to the spinner. When present in the crop there is very little possibility of their effective elimination in ginning, spinning and finishing. "The fuzzy tough integuments of unfertilised seeds are one of the most troublesome impurities of cotton, for they cling persistently and produce numerous 'fuzzy motes' when ground up in the card* ".

Thus the production of cotton free of motes is not only important from the point of view of the spinner, but is also agriculturally important as every mote is a loss to the grower by reason of the loss of lint which it would have borne, if it had developed into a sound seed. The number of motes which are present in a commercial crop are very often overlooked, because ginning seed cotton on the roller gins, as is customary in India, does not reveal this defect as the motes pass on along with the lint. During 1932 one of us (M.A.) had the opportunity of ginning some of his cotton on a saw gin and it was found that the number of motes

* Peirce, F. T., Summary and Proceedings of Conference on Cotton Problems. Empire Cotton Growing Corporation, 1930, P. 11.

which were separated from the lint was simply enormous. Data on the comparative merits of ginning on saw and roller gins are being collected and will be presented separately. The present investigations were, however, started with a view to find out the location of motes in the individual locks of cotton, the nature of the causal factors and methods of elimination, if possible.

Historical.

Harland [1927] discovered that some lethal factors were responsible for the death of developing ovules inside the boll. He has also recorded some varietal differences in the number of motes and has indicated the possibility of producing cotton absolutely free from motes by proper selection. While this may be possible in pedigree cultures in the breeding plot, the chances of obtaining a variety which under field conditions will be absolutely free of this defect seem remote. A smaller number of motes per boll may, perhaps, be produced by very careful selection.

Rea [1928] working in Texas found a definite increase in the percentage of motes from apex to base of locks in all the varieties studied. He also found that the number of motes was higher in 5-lock bolls than in 4-lock bolls and suggested nutritional or pollination defects for this regular distribution. He [1919 ; 1,2] also found that the inherent propensities of different varieties to produce motes varied very much and that seasonal environmental fluctuations produced different reactions in different varieties so far as the number of motes was concerned. No correlation was found between the yield of seed-cotton per plant and the percentage of motes whilst a significant negative correlation was found between the size of bolls and the percentage of motes. Too much importance, however, should not be attached to these correlations until their biological basis has been worked out.

Bailey [1930] carried out some experiments on Sakel and a (fixed) hybrid between Sakel and Sea Island and found that covering the plants by means of mosquito netting to exclude insects did not produce any difference in the percentage of motes per plant. He arrived at the conclusion that " abundant water supply was conducive to a high rate of seed-setting and *vice versa* ". His results also showed that lack of proper pollination was not in any way connected with the occurrence of motes.

Eaton's [1931] experiments with Acala and Pima cottons at Sacaton, Arizona showed that in Pima there was a tendency to produce fewer motes in bolls which were presumably better nourished, but this effect was not noticeable in Acala.

Experiments were designed to find out the ratio of motes to ovules (referred to in the text as percentage of motes) in three strains of Punjab-American cotton at

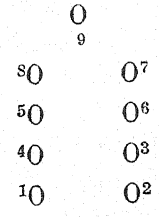
Lyallpur. These will be described in detail. The effect of environment on the production of motes will also be discussed.

Experimental data.

In order to find out the percentage of motes, fifty average plants of each of 4 F, 289 F, and the new Early Strain (*G. hirsutum*) were selected each year at random from the Physiology plots†, Cotton Research Farm, Lyallpur, during September. All the bolls which matured on these plants were collected along with the burr‡ separately for each plant. During 1929, all the bolls were collected at the end of the season, while in 1930 and 1932 the usual three pickings were taken.

During these years the produce from each plant was kept separate for each picking and examined separately with regard to the number of locks per boll, number of ovules per lock and the number and position of motes in the locks.

In order to be able to tabulate systematically the position of motes in the lock of cotton, the position occupied by each seed in the ideal lock was given a number. When the lock was placed on the table with the base of the lock near the observer and the inner surface of the lock uppermost, the left hand bottom seed position was numbered one, the right hand bottom position two, the right hand sub-basal three and so on serially to the solitary seed at the top of the lock as shown in the following diagrams :—



The number of motes was found to be considerable in all the varieties except for the "Early Strain" in 1929. It must, however, be pointed out that this variety was grown very widely spaced in that year and the smaller number of motes in this case may be due to the consequent better nourishment of the plants and the bolls. This variety was grown with normal spacings in 1930 and 1932.

While the actual comparison of the different figures obtained during the three years will be relegated to another section of the paper, they are displayed side by side in Table I.

† Soils of these plots are alluvial loam, fairly rich and very uniform. The cotton crop had practically the same cultural treatments in all years.

‡ The dried-up pericarp of the boll when ripe.

TABLE I.

	4 F			289 F			Early Strain		
	1929-30	1930-31	1932-33	1929-30	1930-31	1932-33	1929-30	1930-31	1932-33
No. of plants examined	50	50	50	50	50	50	50	50	50
No. of bolls examined	509	772	1,488	540	568	1,635	973	648	1,450
No. of bolls with 3 locks	119	177	262	19	13	29	2	10	57
No. of bolls with 4 locks	382	590	1,213	348	383	911	442	378	1,032
No. of bolls with 5 locks	8	5	13	173	172	695	529	260	361
Mean No. of locks per boll	3·7	3·76	3·83	4·3	4·36	4·41	4·54	4·36	4·21
Mean No. of ovules per lock	7·61	7·19	7·09	6·98	6·51	6·71	7·57	6·83	6·84
Mean No. of ovules per boll	28·84	26·03	27·15	29·90	28·38	29·59	34·37	29·78	28·80
Total No. of ovules	14,679	20,084	36,900	16,146	15,294	42,657	33,442	18,205	38,110
Total No. of motes	1,457	2,338	7,259	1,879	2,693	5,471	2,170	5,110	5,325
Percentage of motes	9·90	11·64	19·67	11·63	17·15	12·82	6·49	28·0	14·0

As has already been stated the produce during 1930-31 and 1932-33 was gathered in three pickings. The percentage of motes in these pickings varied very much and are given in the subjoined table.

TABLE II.

Percentage of motes in different pickings.

Variety	Picking number	Percentage of motes	
		1930	1932
4 F	1st . . .	15·1	22·3
	2nd . . .	9·3	15·9
	3rd . . .	8·5	9·3
Average for the whole crop	..	11·64	19·67
289 F	1st . . .	17·60	18·4
	2nd . . .	14·38	12·8
	3rd . . .	..	7·8
Average for the whole crop	..	17·15	12·82
Early Strain	1st . . .	33·9	14·5
	2nd . . .	21·6	12·5
	3rd . . .	21·5	2·5
Average for the whole crop	..	28·0	14·00

It was also observed that of all the plants examined during the three seasons, not one was found entirely free from motes. There was, however, great variation in the number of motes in the individual plants ; the variation ranged from 4·28 per cent. to 25·02 per cent.

This enormous variation in the individual plants may have a selective value provided the plants breed true to this character. This is, however, a point for future investigations.

It was also found that, in individual plants, the percentage of motes from boll to boll and also from lock to lock showed great variation, a range of 0 per cent. to 70 per cent. being observed in the bolls.

Another point of interest in this connection was the variation in the percentage of motes in bolls with three, four and five locks. The percentage of bolls absolutely free of motes decreased as the number of locks per boll increased. This is fully substantiated by figures in Table III.

TABLE III.

Variation in percentage of motes in different types of bolls.

Variety	Bolls with three locks			Bolls with four locks			Bolls with five locks		
	Total number of bolls	Bolls free of motes	Percentage of bolls free of motes	Total number of bolls	Bolls free of motes	Percentage of bolls free of motes	Total number of bolls	Bolls free of motes	Percentage of bolls free of motes
4 F	118	29	24·6	382	70	18·3	8	0	..
289 F	19	4	21·1	348	38	10·9	173	7	4·1
Early Strain	2	1	..	453	100	22·1	534	51	9·6

Not only was a greater percentage of 3-locked bolls as a whole free of motes when compared to 4- or 5-locked bolls, but the percentage of perfect locks was also higher in the bolls with fewer locks, as will be seen from Table IV.

TABLE IV.

Percentage of locks free of motes in different types of bolls.

—	4 F	289 F	Early Strain
Percentage of perfect locks in 3-locked bolls . .	56·5	57·9	..
„ in 4-locked bolls . .	56·4	53·8	67·3
„ in 5-locked bolls . .	..	48·6	57·8

No comment is necessary on the above tables except that it seems highly probable that bolls with fewer locks can for that reason better nourish their developing ovules and hence produce a smaller number of motes.

The position of motes in the lock was very interesting. These were distributed on a definite pattern and the following table illustrates this point.

TABLE V.

Position of motes in locks of cotton.

	Seed position in the lock								
	I	II	III	IV	V	VI	VII	VIII	IX
					4 F				
Percentage of motes in 1929 .	12·7	12·9	9·7	11·1	7·8	8·3	9·2	8·9	7·1
,, 1930 .	19·3	17·8	11·3	10·3	8·2	7·4	7·7	8·2	12·7
,, 1932 .	31·6	31·4	21·0	19·1	13·7	11·6	10·6	10·0	9·6
					289 F				
Percentage of motes in 1929 .	14·1	12·8	10·2	9·6	12·4	10·9	11·2	12·3	12·0
,, 1930 .	23·3	21·2	15·4	15·5	13·4	13·6	17·3	20·8	..
,, 1932 .	18·4	17·1	11·4	10·9	10·0	10·8	10·6	8·7	19·0
					Early Strain				
Percentage of motes in 1929 .	6·5	6·1	6·1	5·6	7·2	7·4	6·8	7·8	4·7
,, 1930 .	28·7	29·6	26·2	23·6	27·5	27·4	33·8	38·1	..
,, 1932 .	20·7	22·6	14·7	12·8	8·8	9·2	8·4	7·4	7·2

All the figures in the above table show the same general trend and the minimum number of motes was situated in the central or near the top seed positions.

During 1930 and 1932 the percentage of motes in the different seed-positions was determined for each picking as well. These figures are comparable with those given in Table V and are shown in the sub-joined table.

TABLE VI.

Percentage of motes in locks of cotton in different pickings.

		Seed-positions in the lock								
		I	II	III	IV	V	VI	VII	VIII	IX
					4 F					
1st picking	1930	24·1	23·8	16·1	14·0	10·4	9·4	9·4	11·3	10·9
	1932	36·0	36·6	24·6	22·7	15·2	14·0	11·6	10·5	10·5
2nd picking	1930	16·4	14·7	8·5	7·6	6·3	6·2	6·4	7·8	13·1
	1932	25·8	25·3	16·0	14·6	11·6	7·1	9·1	7·6	..
3rd picking	1930	14·8	9·7	6·3	8·0	8·1	5·2	7·3	9·4	..
	1932	15·8	11·1	10·8	4·8	7·7	7·8	5·8	8·8	..
					289 F					
1st picking	1930	24·0	22·1	15·7	15·7	13·5	14·3	17·5	22·5	14·3
	1932	25·8	24·0	16·8	15·9	15·8	15·5	13·6	12·1	27·5
2nd picking	1930	18·9	16·0	13·7	15·0	12·6	9·2	16·4	..	..
	1932	20·1	16·0	10·7	11·3	9·0	11·6	10·1	8·7	12·5
3rd picking	1932	9·5	12·0	7·0	5·9	5·9	5·9	8·7	5·0	8·7
					Early Strain					
1st picking	1930	36·7	37·4	32·7	30·8	31·0	31·1	36·8	39·3	46·2
	1932	21·7	23·3	15·2	13·1	9·2	9·7	8·4	7·9	7·4
2nd picking	1930	18·1	19·7	19·3	14·7	24·6	25·0	30·8	33·6	..
	1932	17·9	21·4	13·5	11·8	7·6	7·6	8·5	6·6	..
3rd picking	1930	24·3	24·3	18·0	18·0	21·1	18·6	27·0	..	..

Here again it is seen that the minimum number of motes in each picking was situated in central or top seed position in the locks.

The regular distribution of motes in the different seed-positions in the lock and the variations exhibited by different bolls on the same plant and the different locks in the same boll would seem to preclude any possibility of lethal factors being responsible for their occurrence. The other factors which would cause the production of motes would be :—

(*a*) defective pollination.

(*b*) defective nutrition.

These factors will now be considered separately.

(a) Defective pollination as a cause of mote production.

It seems unlikely that defective pollination can play much part in mote production. Counts made on the stigmas of naturally-pollinated flowers in the field showed without exception that the number of pollen grains was more than sufficient to fertilise all the ovules.

Bailey [1930] in the Sudan showed that :—

" (1) No evidence was obtained of improvement in seed setting as a result of artificial selfing or crossing.

(2) Complete removal of one or more stigma lobes did not prevent fertilisation taking place within the loculi corresponding to the amputated stigma, but the number of fully developed seeds was reduced, the deficiencies being represented by ' blind ' seeds.

(3) When the greater part of all the stigmatic surface was cut off leaving only a very small area of papilliated surface, satisfactory fertilisation still took place. "

It might be thought from this that defective pollination did result in mote production, but in the field, it can be safely assumed that the majority of bolls insufficiently pollinated would be shed. The ultimate crop would thus be gathered from bolls formed mainly from properly pollinated flowers.

Experiments in which flowers of 4 F and 289 F were pollinated with a definite number of pollen grains supply confirmation of the above assumption. These figures are presented in Table VII.

TABLE VII.

Shedding percentage in flowers pollinated with a definite number of pollen grains.

No. of pollen grains put on the stigma.

Name of variety.	1			10			20			30			40			50			60			Ample		
	No. of flowers treated	No. of bolls shed	Shedding percentage	No. of flowers treated	No. of bolls shed	Shedding percentage	No. of flowers treated	No. of bolls shed	Shedding percentage	No. of flowers treated	No. of bolls shed	Shedding percentage	No. of flowers treated	No. of bolls shed	Shedding percentage	No. of flowers treated	No. of bolls shed	Shedding percentage	No. of flowers treated	No. of bolls shed	Shedding percentage	No. of flowers treated	No. of bolls shed	Shedding percentage
4 F	10	10	100	10	10	100	10	8	80	10	9	90	10	8	80	10	7	70	10	5	50	17	9	52·9
289 F	8	8	100	8	8	100	8	7	87·5	8	8	100	8	7	87·5	8	6	75	8	1	12·5	14	2	14·3

In this experiment all the pollen grains were placed on a single lobe of the stigma, but fertilisation was found to take place in all the loculi.

It is thus seen (Table VII) that most of the bolls with defective pollination would be shed. A few of these would, of course, be retained and would be gathered in the crop, but defective pollination must be considered only a minor contributory cause to the production of motes in a field crop. If pollination were defective and the bolls held, an increase in the percentage of motes in those particular bolls would be expected.

(b) Defective nutrition as a cause of mote production.

It may here be mentioned at the outset that the results so far obtained point to defective nutrition being the primary cause influencing mote production.

During 1930, twenty-five plants of 4 F were grown in similarly-sized drums of uniform soil, and were given different amounts of water and different manurial treatments. The percentage of motes in each series of five plants is shown in Table VIII.

TABLE VIII.

Percentage of motes in 4 F plants differently treated.

Treatment	Percentage of motes to ovules
Normal watering	18·6
Twice normal watering	14·3
Manured with ammonium sulphate at 2 maunds per acre	12·7
„ „ potassium sulphate at 2 maunds per acre	6·6
Average of manured plants	10·4

From the above table, it may be inferred that abundant watering was conducive to the production of a smaller percentage of motes and that the application of artificial manures also decreased their production. The effect of potash manure is very marked, and since potassium salts are believed to affect photosynthesis favourably, the interest in the above figures is patent. The Pubjab soils are, however, known to have abundant available potash and though field scale manurial experiments at the Lyallpur Agricultural Farm with this manure have not given

any positive results, yet the above figures are not without interest, as the plants grown in these drums were limited in the soil mass available, and actually did not make as good growth in shoot and root as plants grown in the field. Since the number of plants was small, the figures may not be statistically significant.

Also the Early Strain grown wide-spaced in 1929 produced very few motes as compared to the same variety grown with normal spacings in 1930 and 1932 (Table I). The individual plants in 1929 grew very big and produced abundant crop due to the better nourishment of the plants.

Moreover it is a matter of common observation that in heavy bearing plants bolls borne near the tips of sympodia open less well than bolls borne near the main stem showing thereby the extent of competition for nutrients between bolls on the same branch. It has also been ascertained that the badly-opened bolls contain a higher percentage of motes as compared to the well-opened ones as the following figures taken on another occasion will show.

TABLE IX.

Percentage of motes in well-opened and badly-opened bolls.

	4 F	289 F	Early Strain
Well opened bolls	5·2	6·2	4·2
Badly opened bolls	5·8	7·4	8·4

From the foregoing results it may be inferred that if the plant is amply provided with nutrients and if there is ample amount of water available, fewer motes will be produced. A great deal of microscopic work on the development of motes has also been done and will be presented in a separate paper. It may, however, be mentioned here that all the motes examined showed considerable development of various tissues. Motes must, therefore, be considered as fertilised ovules which have failed to develop into seeds and not as the remains of unfertilised ovules.

Thus there is a very great probability that differences in mote production are due to the physiological response of the plant to its environment. The defective nutrition of bolls which results, in consequence of these differences in physiological response, may be modified one way or the other by the following factors :—

(*i*) The distribution and size of the vascular bundles in the boll.

(*ii*) The rate of flow of nutrients to the different portions of the boll.

(*iii*) The internal pressure set up by a possibly differential rate of growth of locks and the epicarp of the bolls.

(*iv*) The competition for nutrients between different seeds inside the boll.

The effect on environment on mote production will now be considered.

The effect of environment has been suggested to be one of the chief causes of neppiness in cotton and the production of motes by Peirce [1930], Clegg [1930], Rea [1929], and other workers. Soil and climate are the chief components of 'environment', but in the present case, the effect of soil has, as far as possible, been eliminated by using a very uniform piece of land similarly cultivated during the course of this experiment.

To investigate the effect of environment advantage was taken of the sowing date experiments carried out at Lyallpur. The results obtained from these experiments are interesting.

Before describing the experimental details it may be of interest to enumerate the climatic conditions which obtained at Lyallpur during 1929 and 1930. Out of the different factors which jointly constitute the 'climate' only the following main factors will be considered and are shown in Figs. 1 and 2.

(i) Maximum temperature.
(ii) Minimum ,,
(iii) Relative humidity.
(iv) Rainfall.
(v) Number of rainy days.

An examination of Figs. 1 and 2 will reveal that there were not very vast differences in the climatic conditions of 1929 and 1930. The maximum and minimum temperature records, however, show that 1930, should be considered as a somewhat 'milder' year than 1929.

The sowing date experiments will now be considered in detail, and as the dates of sowing in the two years were different, the two experiments will be dealt with separately.

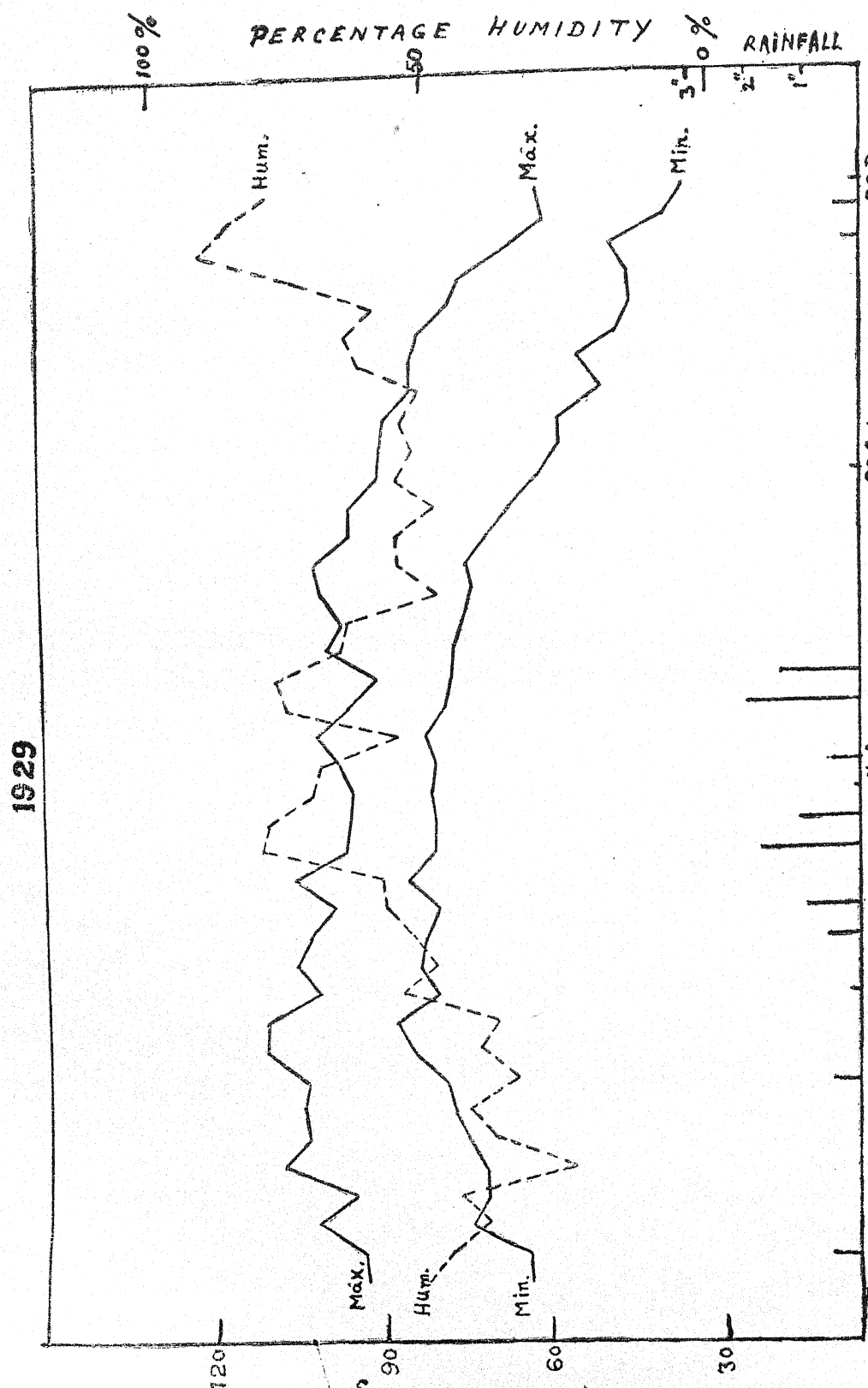

PERCENTAGE HUMIDITY
100 %
50
0 %
RAINFALL
3"
2"
1"
Hum.
Max.
Min.
1929
29 Dec
20 Oct.
11 Aug.
2 June
Week ending
Max.
Hum.
Min.
120
90
60
30
Temperature in Deg. F.

Fig. 1.

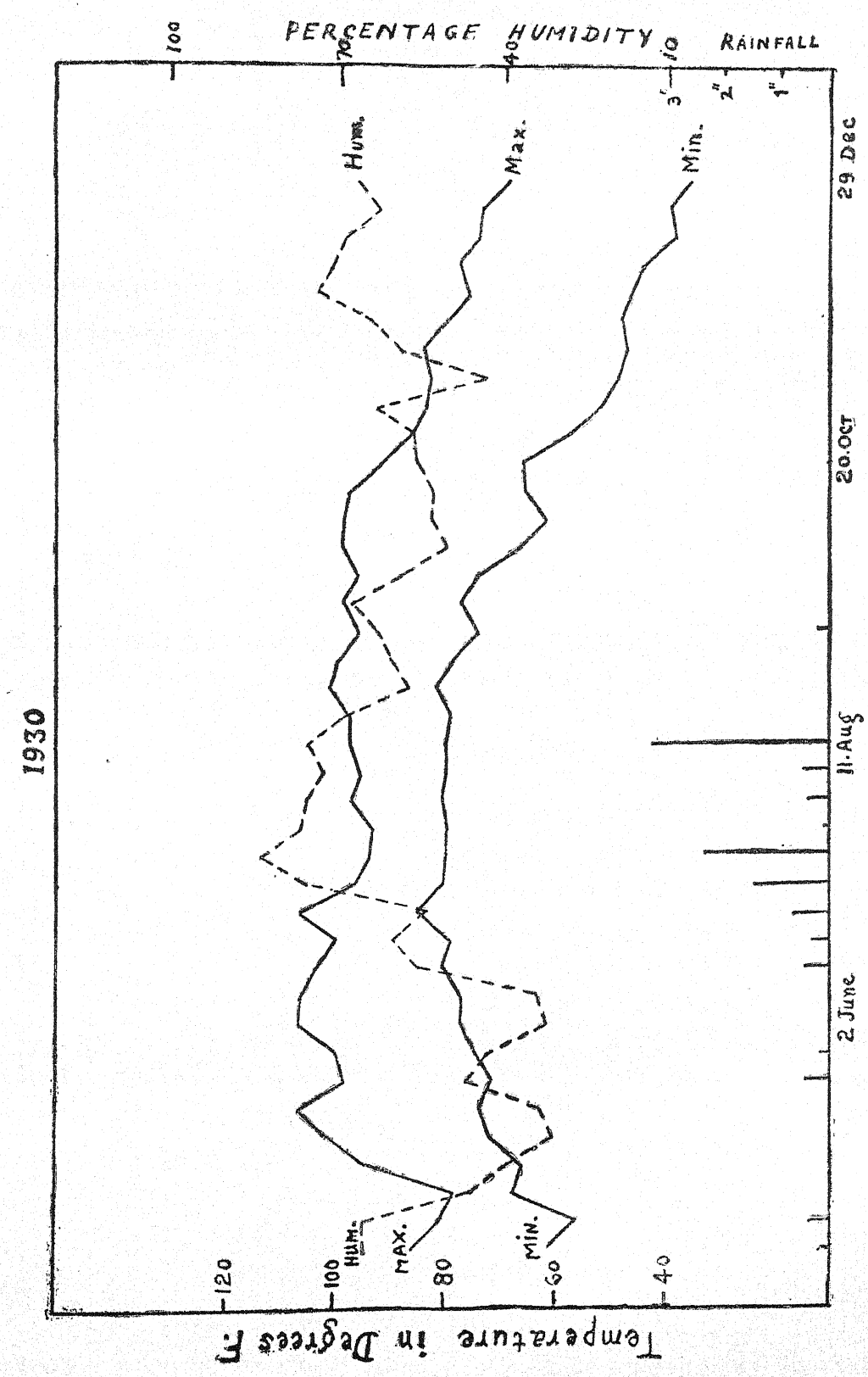

PERCENTAGE HUMIDITY
RAINFALL
100
70
40
10
3"
2"
1"
Hum.
Max.
Min.
29 Dec
20 Oct
11 Aug
2 June
Week endings
1930
Temperature in Degrees F.
120
100
80
60
40
Hum.
Max.
Min.

Fig. 2.

(a) Sowing date experiment, 1929.

There were five dates of sowing, namely 1st May, 15th May, 31st May, 15th June and 1st July with ten repetitions of each sowing. The variety employed was 4 F.

As the plants of different sowings would grow and mature under different climatic conditions, the differences in the percentage of motes, if any, could be taken to be due to the effect of environment.

Trought [1930] has analysed the different factors which are conducive to higher yields in late-sown 4 F and has cited the experience of Roberts [1929, 1, 2] at the British Cotton Growing Association Farm, Khanewal, where also late sowings have been profitable on a large commercial scale. He suggests that "differences from the optimum between the roots' and shoots' environment may account for differences between different sowing dates by inducing a suboptimal root/shoot, ratio, which has a cumulative effect throughout development".

If the cause of more mote production is defective nutrition, as has been suggested earlier, the figures from the sowing date experiment would be highly interesting.

Fifty average plants, five from each of the ten repetitions, were selected at random from the five dates of sowing in September 1929 and all the produce was gathered separately for each plant. The number of motes was calculated and the figures are given below :—

TABLE X.

	1st sow 1st May 1929	2nd sow 15th May 1929	3rd sow 31st May 1929	4th sow 15th June 1929	5th sow 1st July 1929
No. of plants examined	50	50	49	50	50
„ bolls „	572	474	538	532	525
„ locks „	2,178	1,806	1,882	2,082	2,031
No. of bolls with 3 locks	116	97	76	54	62
„ „ „ 4 „	450	370	456	453	437
„ „ „ 5 „	6	7	6	25	26
Mean No. of locks per boll	3·8	3·8	3·9	3·9	3·9
„ „ ovules per lock	7·73± 0·02	7·60± 0·03	7·67± 0·03	7·50± 0·02	7·21± 0·03
„ „ „ „ boll	29·37	28·88	29·91	29·25	28·22
Total No. of ovules	16,836	13,726	14,438	15,600	14,644
„ „ motes	1,708	1,339	1,230	794	704
Percentage of motes	10·14	9·75	8·52	5·09	4·81

It will be seen from figures in Table X that the production of motes was affected considerably by the date of sowing of the crop; the number of motes being considerably reduced with later sowings. This point is further emphasized by the following figures showing the percentage of bolls absolutely free of motes, and the percentage of perfect locks for each sowing:—

TABLE XI.

Percentage of bolls with no motes in different sowings.

—	Bolls with 3 locks			Bolls with 4 locks			Bolls with 5 locks		
	Total No. of bolls	Bolls with no motes	Per-centage	Total No. of bolls	Bolls with no motes	Per-centage	Total No. of bolls	Bolls with no motes	Per-centage
1st sow . .	116	30	25·86	450	59	13·11	6	2	..
2nd sow .	97	33	34·02	370	97	26·21	7	1	..
3rd sow .	76	34	44·73	456	111	24·34	6	0	..
4th sow .	54	26	48·14	443	150	33·86	24	3	12·50
5th sow .	61	40	65·57	437	144	37·95	16	3	18·75

TABLE XII.

Percentage of perfect locks from different sowings.

——	1st sow	2nd sow	3rd sow	4th sow	5th sow
Percentage of perfect locks (3-lock bolls)	55·2	58·6	69·7	79·0	80·9
Percentage of perfect locks (4-lock bolls)	53·5	59·7	63·7	73·9	73·5
Percentage of perfect locks (5-lock bolls)	..	..	..	65·8	71·2

From the data presented above, it will be clear that late-sown plants owing to their better capacity for nourishment [Trought, 1930] produced a smaller number of motes.

The distribution of motes in the locks of cotton follow much the same line as in the three varieties already mentioned in Table V, as will be seen from the following table.

TABLE XIII.

Percentage of motes in different seed-positions.

—	Position of ovules and motes in the lock								
	I	II	III	IV	V	VI	VII	VIII	IX
1st sow									
No. of ovules .	2,181	2,181	2,181	2,181	2,180	2,175	2,099	1,066	200
No. of motes .	292	254	224	204	219	181	195	114	25
Percentage of motes.	13·4	11·7	10·3	9·4	10·0	8·3	9·3	10·7	12·5
2nd sow									
No. of ovules .	1,806	1,806	1,806	1,805	1,804	1,795	1,702	745	117
No. of motes .	231	215	182	207	159	146	144	49	6
Percentage of motes.	12·8	11·8	10·1	11·5	8·8	8·1	8·4	6·6	5·1
3rd sow									
No. of ovules .	2,080	2,080	2,079	2,079	2,079	2,074	1,997	977	169
No. of motes .	225	221	165	160	129	129	126	69	15
Percentage of motes.	10·8	10·7	7·9	7·7	6·2	6·2	6·3	6·1	9·0
4th sow									
No. of ovules .	2,107	2,107	2,107	2,107	2,107	2,101	2,003	670	94
No. of motes .	148	163	94	88	82	81	81	43	13
Percentage of motes.	7·0	7·7	4·5	4·2	3·9	3·9	4·0	6·4	13·8
5th sow									
No. of ovules	2,031	2,031	2,031	2,029	2,029	2,004	1,714	423	32
No. of motes .	131	160	93	96	60	77	68	16	3
Percentage of motes.	6·4	7·8	4·6	4·7	2·9	3·8	3·9	3·8	9·4

(b) Sowing date experiment, 1930.

During 1930, three dates of sowing, namely 24th March, 1st May, and 9th June, were used and there were six repetitions of each sowing. The variety employed was again 4 F.

Fifty average plants were selected at random from each sowing and the usual three pickings were taken. The produce of each plant was kept separate for every picking. The number of motes was calculated for each plant separately and the figures are tabulated below :—

TABLE XIV.

	1st sow	2nd sow	3rd sow
No. of plants examined	50	50	50
„ bolls „	573	772	650
„ locks „	2,154	2,916	2,628
No. of bolls with 3 locks	111	177	20
„ „ „ 4 „	451	590	582
„ „ „ 5 „	11	5	48
Mean No. of locks per boll	3·82±0·02	3·76±0·02	4·00±0·01
„ „ ovules „ lock	7·17±0·02	7·19±0·02	7·34±0·015
„ „ „ „ boll	27·39±0·01	26·03±0·17	29·36±0·01
Total No. of ovules	15,453	20,084	18,884
„ „ motes	2,847	2,338	2,131
Percentage of motes	18·4	11·64	11·28

Comparing the figures obtained in 1930 with those for 1929, it is found that there is quite a good deal of agreement, and the percentage of motes decreased as the sowing time was delayed. The percentage of motes, however, did not decrease in the same way as in 1929. The sowing date experiment in 1930 has been described elsewhere [Trought, Afzal and Iyer, 1931] and it was suggested that because the 1930 season was milder than 1929, the advantages of late sowing were

obscured to some extent in this year. The checks to growth which the early sown 4 F cotton was expected to suffer during a normal May having being absent, cotton sown early gave a higher yield than the late sown cotton in this year, as it had a longer period of growth. The first date of sowing, namely 24th March appears to be too early, however, even in mild years.

It may therefore be stated that, presumably, owing to their better capacity for nourishing their bolls, the late sown 4 F plants produced a smaller number of motes as compared with the earlier sown plants. The position of motes in the lock also followed the same line as will be seen from Table XV.

TABLE XV.

Position of motes in the locks of 4 F. Sowing date experiment 1930.

Seed positions in the lock.								
I	II	III	IV	V	VI	VII	VIII	IX
1st sow								
27·6	25·4	17·9	17·2	13·1	13·4	14·7	14·3	20·6
2nd sow								
19·3	17·8	11·3	10·3	8·2	7·4	7·7	8·2	12·7
3rd sow								
17·0	16·5	9·7	11·0	7·9	8·6	8·0	10·5	10·0

Now taking all the data of the sowing date experiment for both years into consideration, it is abundantly clear that 4 F goes on improving so far as the number of motes is concerned as the sowing time is delayed.

The preliminary data on the response of 4 F to the sowing date so far as yield is concerned have already been published [Trought, 1930 and Trought, Afzal and Iyer, 1931] and a more detailed examination of this problem has now been completed and will be published shortly.

SUMMARY.

From the data presented, there is evidence that the most important cause of the production of motes is to be found in a nutritional defect of individual bolls and

that defective fertilisation plays a very minor part. The presence of lethal factors in the Punjab-American cotton strains is not indicated by the data obtained so far.

The motes are located on a definite pattern in the lock. The middle or the top of the lock has the least number of motes.

The data from the sowing date experiments have shown that in the case of 4 F, the number of motes went on decreasing as the sowing time was delayed.

ACKNOWLEDGMENTS.

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REVIEW

The culture and marketing of tea. By C. R. HARLER, Ph.D., B.Sc. F.I.C., Pp. XII+389, 8 Illustrations and 2 Maps. (London: Oxford University Press, 1933) Rs. 8.

If the English literature extant on tea be surveyed, it will be noted that it does not include one volume which is devoted to a general description of the cultural and manufacture methods employed in the main tea producing countries. The tendency is towards exclusiveness of treatment. Thus Watt and Mann's book (unfortunately, now out of print) and that of C. Bald deal mainly with tea in North East India, whilst Whitehead and Elliot limit the scope of their publication to Ceylon.

A book, therefore, reproducing for the general reader an account of cultural methods operative in the main countries engaged in tea production should represent a welcome and important augmentation of tea literature. Dr. Harler's book is an attempt to supply such an addition. The only other book covering even approximately the same ground is a small publication by A. Ibbetson, but this is now somewhat out of date. In any case Dr. Harler's treatment of the subject is rather different, in that he writes from the scientific and practical perspectives, rather than from the commercial point of view.

The author does not claim too much for his effort. He intimates clearly in the prefatory note, that he does not write authoritatively on tea in countries such as Ceylon, Java, China and Japan, which he visited only as an interested spectator. The space occupied in the book by his description of tea in North East India demonstrates his ability to be more comprehensive and his fourteen years' experience in that area provides him with ample qualification.

Most of the material contained in the book has already appeared in print under the author's name and in this sense it cannot be considered as an original contribution. A considerable amount has been reproduced, in a modified form, from the Scientific Journals of the Indian Tea Association, and from articles appearing periodically in the Tea and Coffee Trade Journal. This in no way disparages the value of the book; indeed, it thus becomes a very useful compilation of articles on tea from journals which are not easily available to most readers.

The subject matter of the book is divided into four parts. Part I (78 pages) portrays the tea plant as a botanical unit, and outlines the climatic and soil charac-

teristics upon which its development and cropping capacity depend. Details of the various treatments applied to the tea plant to enable it to fulfil the purpose for which it is cultivated are given, the section ending with a brief description of the procedures adopted to produce the portable commodity from the raw material.

Part II (73 pages) is devoted to the chemistry and pharmacology of tea. Though the significance of the chapters included in this portion of the book may not be apparent to the layman, nevertheless they are an important, indeed an essential inclusion in a general book on tea. The author delineates his scientific views on the main constituents of the leaf, views upon which he must be prepared to base his interpretation of the phenomena occurring during manufacture, and also his conceptions of the ultimate condition in which they are to be found when contributing the most desirable features of the beverage.

Part III (207 pages) represents the major portion of the book. In it the author describes in detail tea as grown, cultivated and manufactured in the principal tea producing countries. This part will therefore undoubtedly offer the greatest attraction to the practical planter.

Part IV (25 pages) includes a chapter on the marketing of tea, which, though not a direct concern of the majority of planters, nevertheless presents an interesting insight into the treatment of tea after it has left the estate and has reached the hands of the brokers and blenders. The concluding chapter of the book introduces statistical data of tea production, export, consumption and prices. It might possibly have been advisable to attach these latter figures in the form of an appendix rather than include them in the main body of the book.

The general trend of the four parts having been outlined in brief, it is of interest to consider each one in greater detail.

In the opening chapter an account is given of the views of various authorities on the still debatable question of the original habitat of the tea plant. It is particularly to be noted that the author reverts to the name given originally to the tea plant by Linnaeus *viz. Thea sinensis*, and rejects the customary name *Camellia Thea*. Dr. Harler's contention for reverting to the old term *Thea sinensis* is based on the international rule governing the naming of plants which states that when two or more groups are united the name of the oldest is retained. It must however be recollected that Linnaeus himself subsequently repudiated the name *Thea sinensis*, and another authority, Sims, was ultimately persuaded of the necessity for reducing the term 'Thea' to 'Camellia'. As Sir George Watt writes 'It would seem that the specific name (*i.e. Thea sinensis*) cannot be given as a collective appellation for the many races of the cultivated tea plant, since it

was rejected by Linnaeus and, as Seeman points out, is geographically hardly correct'. It would, therefore, appear unfortunate that the author should resuscitate a controversial point, which, as far as British systematists are concerned, had been satisfactorily concluded by the classification assigned to the cultivated tea plant by Sir George Watt in a paper read before the Royal Horticultural Society in 1907. It seems improbable in addition that such an eminent systematist as Sir George Watt would have ignored without adequate reason, the rule quoted by Dr. Harler, or that C. S. Cohen Stuart in Java should choose in 1919 to support the generic name of 'Camellia'.

In Chapter I some paragraphs of interest are included which deal with the investigations carried out in Java on the selection of tea, from which the independence of cropping capacity on jat appears to have been a significant, if disconcerting deduction.

A comparative survey of climatological conditions existing in the various tea countries provides the author with the evidence on which he bases inferences with regard to the circumstances of rainfall and temperature which are calculated to sustain economic tea cultivation. Correlations of temperature and humidity as factors regulating cropping potentialities and quality production are indicated.

A chapter is devoted to the characterisation of soils, and the necessity of introducing the effect of climate in any soil classification. The formation of lateritic soils is discussed, and the inter-relationships, between iron and alumina possessing a bearing on the degree of laterisation are demonstrated. The importance of soil acidity as a factor controlling the growth of tea is stressed. If the definitions of 'acidities' are expressed in somewhat lax scientific language the paragraphs should serve to convey in simple phraseology, the practical significance of soil reaction.

The author proceeds in the final chapter of Part I to give a general description of the treatment of the tea plant from the initial stage in which it is planted to a survey of various methods of pruning intended to check the natural upward growth of the plant and maintain it in a shape conformable with ease of plucking combined with greatest plucking area. Part I ends with an outline of the methods employed in preparing tea. As these methods are discussed in greater detail in the chapters devoted to the individual tea producing countries, these paragraphs may be considered as introductory in character.

In Part II the author enters the sphere of scientific investigation on tea, and primarily gives the reader his views on the chemical constituents of the leaf, their influence on the market value of tea, and the changes occurring in them during the process of manufacture.

The views given whether right or wrong, represent the author's conceptions of the fundamentals underlying the whole superstructure of the scientific explanation of manufacture. To the scientific reader, therefore, these chapters acquire an added significance.

The reviewer is unwilling to concede the author's assertion that his conceptions of the chemistry of tea 'express the generally accepted views on the subject'. As one example, the contentions regarding the fate of tannin during manufacture may be cited. On this subject there are two schools of thought, the North East Indian and the South Indian. The former considers that about 6 per cent. of 'oxidised' tannin is retained in an insoluble form by the proteins in the leaf, whilst the latter considers that the tannin molecule sustains the simple addition of oxygen (oxygenation) which does not affect its solubility as regards extraction by hot water. Dr. Harler holds the former view.

Since considerable emphasis is placed by the author on the regulation of this loss of tannin during manufacture, the evidence on which he bases his conclusion merits closer examination.

On page 89 of the book a Table is given indicating the percentage change in the ingredients of green leaf during its transition to black tea. The percentage of 'tea tannin' decreases from 22 to 12. But the '12 per cent.' figure was obtained by taking the average of two values determined by two admittedly unreliable methods of tannin assay (page 105) and hence is not a figure which could reasonably expect scientific acceptance. Furthermore, if the soluble proteins of the leaf are responsible for the decrease in soluble tannin, it might logically be anticipated that there would be a decrease in the water soluble protein. Actually the figures reproduced in the Table show an increase in water soluble protein. It would appear therefore that the evidence for a decrease in tannin during manufacture is inadequate. It might be added that South Indian workers have found no appreciable decrease in the tannin during manufacture, and have established this by the employment of two independent, accurately standardised methods of estimation.

As a second example of a view supported by the author which does not receive universal acceptance the formation of insoluble condensation and 'oxidisation' products may be quoted. The theory of the formation of these products during manufacture was first propounded by Dr. J. B. Beuss in Java to explain the formation of colour in a tea infusion. That such insoluble derivatives of 'tea tannin' may be prepared is to be expected from the constitutional characteristics of 'tea tannin'. Furthermore their formation may easily be demonstrated *in virtro*. Such a demonstration however requires the use of boiling mineral acids over an

extended period, or the employment of strong oxidising agents. It is inconceivable that such products could be formed during a 'fermentation' at low temperature lasting over a period of some 3½ hours followed by approximately 25 minutes desiccation at temperatures usually under boiling point. A further weakness in the theory is to be found in the fact that no such insoluble condensation or 'oxidation' products have been isolated from tea by the use of simple extraction methods.

The foregoing limited criticisms of the author's chemical views must suffice for the purposes of this review.

The third chapter in the chemical section deals with the changes occurring during tea manufacture in the light of the opinions held by Dr. Harler, whilst the forth deals with the pharmacology of tea. This last chapter in Part III would appear to meet most of the animadversions directed at the beverage by dieticians. All scientific investigators with a knowledge of tea chemistry would support Dr. Harler's conclusion of the harmlessness of tea infusions taken in reasonable amounts. At the same time such support would not be forthcoming on the exceedingly flimsy claims of tea tannin to be a pseudotannin or on the production of phlobaphenes, but on the more powerful premises of the combination of 'tea tannin' with caffeine.

The presence of such a compound of 'tea tannin' with caffeins in a tea infusion is not recognised by Dr. Harler but South Indian workers have succeeded in isolating it from green leaf and in its soluble oxygenated form, from Black tea.

The scientific reviewer can only end his comments on Part II with a note of regret that the author of a book which was intended to give general views on the subject of tea, should not have included a careful and critical examination of the chemical conclusions arrived at by other investigators in this field.

Part III of the book embodies an exceptionally interesting and instructive series of chapters dealing with tea in the various tea-producing countries of the world. It is a section of the book which undoubtedly reveals Dr. Harler at his best.

The separate accounts of tea in China and Japan are full of interesting items commencing with the historical outline of tea in China down to the production of scented teas and brick tea, and to a description of the impressive Japanese tea ceremony. It would seem that grading in other tea planting countries has not yet reached the peak of complexity when one reads that in a recent classification of China teas 64 classes of black, and 48 of green teas were distinguished. One reads with no little surprise that in the Shizuoka (Japan) district some 4,000 lbs. tea per acre has been obtained, though the author hastens to add that such is exceptional, but 2,800 lbs. per acre may be expected in the neighbourhood of Uji.

The description of tea in North East India commences with Chapter XI and occupies four chapters totalling over 100 pages of the book. The author divests himself of any proclivities towards restraint which he may have felt in his descriptions of those countries of which he possessed only limited experience ; he assumes the role of an authority and deals with tea in North East India accordingly.

Chapter XI deals with the geomorphological characteristics, the people and the communications of North East India. Chapter XII gives an outline of the development of the tea industry there, this being succeeded by a detailed explanation of the cultural methods employed. If any criticism of these chapters is possible it is the interpolation of Chapter XII between the natural sequence of Chapters XI and XIII.

The representation of the manufacture of tea in North East India will prove interesting reading to tea planters,—especially at the present moment when the question obsessing all minds is the manner in which quality may be improved. Dr. Harler's views on controlled withering combined with the doubts expressed in Ceylon and experienced in Assam as to its utility, may be disconcerting to Planter readers imbued with the idea of regulating wither. It must be remembered however, that Dr. Harler is dealing with this aspect of tea manufacture purely from North East Indian standards.

Among other items of interest included in the description of manufacture, Dr. Harler expresses his views on the advisability of employing 'electrolytic chlorine' in factories. The benefits to be derived from this cleansing agent are decisively negatived by the author and his comments should receive the careful consideration of planters contemplating its use.

One of the most valuable features of the chapter on manufacture is the correlation between the effects produced by any particular condition of withering, 'fermentation', and firing, and the nomenclature employed by tea tasters in describing these effects. In the description of each stage, terms used by tea tasters are printed in italics and a glossary of these terms is given in the succeeding chapter. A planter is thus enabled to trace any undesirable or desirable characteristic in his teas commented on by the tea taster back to the particular stage in manufacture responsible for its production.

Chapter XVI is devoted to tea in other parts of India, Ranchi, Dehra Dun, the Kangra Valley and South India.

The final two chapters of Part III deal with tea in Ceylon and Java. The paragraphs on Ceylon appear to be considerably abbreviated, but this was probably intentional on the part of the author, in view of the recent second edition of White-

head and Elliot's " Tea Planting in Ceylon ". In discussing tea in Java, Dr. Harler has introduced a description of the tea soils together with details of planting, pruning and plucking, manuring and cultivation. A brief reference to manufacture is made with a commentary on the characteristics of Java tea.

The book itself is well printed in large clear types ; certain misprints occur such as on page 114, line 13 where ' Tannin ' should read ' caffeine ' and on page 299, line 10 where ' 6 ' should be ' 5 ' ; and many errors in the spelling of Entomological names could have been avoided. The foregoing points however do not detract from the general readable value of the book, especially at the attractively low price of Rs. 8 for nearly 400 pages of information. (W. S. S.)

APPENDIX

INSTRUCTIONS TO AUTHORS OF PUBLICATIONS OF THE IMPERIAL COUNCIL OF AGRICULTURAL RESEARCH.*

1. All manuscripts should be clean, clear and carefully revised. Only one side of the paper should be used, and as far as practicable the original type-written copy and not a carbon copy should be sent. Capitals should be sparingly used, and all the necessary punctuation should be done in the MS. and not left for introduction in proofs.

2. The title of a paper should not be lengthy.

3. It is desirable that the MS. should have suitable heads and sub-heads. In numbering the principal divisions of a paper roman numerals should be used. The use of arabic figures and (*a*), (*b*), (*c*), etc., is generally reserved for numbering the sub-divisions coming under each head.

4. Articles submitted for publication either in the *Indian Journal of Agricultural Science* or in the *Indian Journal of Veterinary Science and Animal Husbandry* should be accompanied by abstracts for publication in *Agriculture and Livestock in India*. Abstracts should be concise, but should be long enough to explain the matter dealt with; ordinarily no abstract should exceed 200 words.

5. When a word or line is intended to be printed in *italics* it should be underlined with a single line, in SM. CAP. with two lines, in CAPITALS with three lines, and when in **Antique** (heavy type) with a wavy line (~~~~~).

6. In descriptive matter, numbers under 100 and all numbers occurring at the beginning of a sentence should be in words.

7. Local names for crops, technical operations, etc., should be defined where they first occur in the text, *e.g.*, *rabi* (spring crop). The use of local weights and measures should be avoided as far as possible. Vernacular names, such as *jowar*, *bajri*, should be in italics without a capital letter, and each such name where it first occurs should be followed by its scientific equivalent in brackets, *e.g.*, *jowar* (*Andropogon Sorghum*). It is usual to write the initial letters of varietal names in capitals, *e.g.*, Striped Mauritius, Dharwar-American cotton and Broach cotton.

8. Botanical and zoological names are printed in italics and should be underlined in the MS., *e.g.*, *Triticum vulgare* L.; *Diplodia Corchori* Syd.; *Pyrilla aberrans*

* Spare copies of these Instructions can be had on application to the Secretary, Imperial Council of Agricultural Research (Publication Section), New Delhi.

Kirby. The International Rules of Botanical Nomenclature and the International Rules of Zoological Nomenclature should be followed. The names of chemical substances should not be written with a capital letter; they are printed in roman type (*e.g.*, calcium carbonate, prussic acid).

9. The following and similar abbreviations may be used freely :—*viz.*, *e.g.*, *i.e.*, mm. (millimetre), cm. (centimetre), grm. (gramme), mg. (milligramme), c.c. (cubic centimetre), sp. gr. (specific gravity), lb. (pound), cwt. (hundredweight), in. (inch) ft. (foot), oz. (ounce), md. (maund), sr. (seer), ch. (chattak). Other abbreviations should be used sparingly, if at all.

10. References to plates should be given within brackets, without prefixing the word " see " or " cf.", in the MS. itself, and should not be left over for introduction in proofs. For exmple, " The parasite (Pl. X, fig. 4) was present late in 1906."

11. The word " Table " is preferable to " Statement ", and tables should be numbered consecutively in roman figures. Each table should have an explanation as a sub-head. It is more convenient for reference if tables can be printed horizontally; for this purpose they should not exceed in width the printing measure of the page (5″). *Example—*

TABLE IV.

Results of water-saving experiments on wheat (Pusa 12) at Gungapur, Haripur and Sargodha, 1916-17.

Station	No. of irrigations including the preliminary watering	Yield per acre in maunds and seers		Average yield per acre	
		Grain	Straw	Grain	Straw
		Mds. Srs.	Mds. Srs.	Mds. Srs.	Mds. Srs.
Gungapur	One	12 19½	20 10	9 34	21 17
Haripur	"	8 31	19 14		
Sargodha	"	8 12½	25 27½		

12. References to literature, arranged alphabetically according to authors' names, should be placed at the end of the article, the various references to each author being arranged chronologically. Each reference should contain the name of the author

(with initials), the year of publication, the abbreviated title of the publication, volume and page. In the text the reference should be indicated by the author's name followed by the year of publication enclosed in brackets; when the author's name occurs in the text, the year of publication only need be given in brackets. If reference is made to several articles published by one author in a single year, these should be numbered in sequence and the number quoted after the year both in the text and in the collected references. This system of referencing is the same as is used in the *Biochemical Journal* with slight modification and will be clear from the following illustration :—

The work of Osborne and Mendel [1919, 1, 2] and Steenbock and Boutwell [1919] had indicated an association of the fat-soluble vitamin with the green parts of plants. This view was examined by Coward and Drummond [1921] who reported that vitamin A was not synthesised by etiolated shoots but that green leaves were active in its formation. Another worker [Wilson, 1922], on the other hand, found that etiolated shoots if given in sufficient quantity could supply the fat-soluble vitamin and that this factor was therefore formed in the absence of light.

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Abbreviations, as far as possible, should follow the system adopted in " A World List of Scientific Periodicals " published by the Oxford University Press.

13. Papers should be complete when submitted for publication. As alterations and additions at the proof stage cause both additional expense and delay, they should be resorted to as little as possible. In making corrections in proofs the recognized symbols which will be found in the " Standard Dictionary " should be used. Second (page) proofs will be submitted to authors who should return them promptly.

Illustrations.

14. As the *format* of the journals has been standardized, the size adopted being crown quarto (about $7\frac{1}{8}'' \times 9\frac{5}{8}''$ cut), no text-figure when printed, should exceed $4\frac{1}{2} \times 5$ inches. Figures for plates should be so planned as to fill a crown quarto plate—the maximum space available for figures being $5\frac{3}{4} \times 8$ inches exclusive of that for letter press printing.

15. Photos or drawings for illustration should accompany the manuscript and each should bear on the reverse side the name of the paper to which it relates together with the title or legend, figure or plate number, and the size to be reproduced. When giving instructions for reduction linear measurements are under-

stood ; thus, " half-size " means reduce to half the length and breadth, not half the area. A photograph should not be rolled up, nor pinned, and should always be packed flat. A complete list of plates and figures should always accompany the paper.

16. Line drawings should be made with clear black lines on smooth white paper, preferably Bristol board. Rough paper should be avoided. Care should be taken that all the lines are drawn firmly ; scratchy or grey lines, produced by the ink being thinned down, are not permissible. Drawings should be larger than the required size. All lettering should be neatly and clearly put in, care being taken to make all lettering sufficiently large to stand reduction.

17. For half-tone work, copy should be made on glossy silver paper and of the same size or larger than the size required.

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19. For detailed instructions regarding preparation of illustrations, it would be of advantage to refer to Mr. C. M. Hutchinson's article on " Photographic Illustrations " in the *Agricultural Journal of India*, Vol. XI, Pt. 3, July 1916, and Mr. A. W. Slater's paper on " The Preparation and Reproduction of Scientific Illustrations " in the *Proceedings of the Third Entomological Meeting*, 1919, which has been reprinted as *Bulletin No. 114 of the Agricultural Research Institute, Pusa.*

ORIGINAL ARTICLES

FOOT-ROT DISEASES OF *PIPER BETLE* L. IN BENGAL.

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(With one text-figure)

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Introduction.

The cultivation of the betel-vine (*Piper betle* L.) in lower Bengal as elsewhere in this country is restricted to a definite community and the knowledge of cultural methods is passed on from father to son. The '*baroi*', as such a cultivator is called, has well defined rules as to what should be done and what avoided. He grows the vines in a '*boroj*' which is a kind of hut whose sides and roof are usually composed of reeds, jute stalks, or grass on a light frame-work of bamboo. The vines are grown in rows on ridges and tied to jute stalks for support. The *boroj* may be a few square yards or extend up to as much as a quarter of an acre and several *borojes* are often placed side by side covering a large area. The air inside the hut is always moist and still, and during the monsoon saturated. The water level at this time comes very near the surface and in places the surface of the soil is awash for considerable periods. In circumstances like these great care has to be exercised in growing the vines and successful cultivation thus becomes a tricky business. Even mildly pathogenic fungi that have access to the vines may cause considerable damage. It is perhaps this fact that has led the *baroi* rigorously

to exclude from his *borojes* all persons who are not members of his family or dependents, even though his explanation of the restriction is somewhat different. Experience has shown him that disease may be brought to his *boroj* by human agency and he has acted in the best way to protect himself. In such circumstances it comes about that loss from disease is not heard of unless it assumes proportions much greater than usual and it is therefore very difficult to obtain accurate information, for seldom indeed is permission to examine the plants ever granted.

THE DISEASE.

As early as 1885 betel-vine diseases seem to have attracted the attention of the revenue officers of Government, for on page 57 of the report of the Department of Land Records and Agriculture, Bengal, 1885-1886, mention is made of a "disease of leaves called Chittabari in which the edges of the leaves rot" and of a "disease of the betel-vine stem in which the joints of the plant turn black and then rot". The infectious nature of the disease seems to have been suspected even at that time and the practice of carefully removing the diseased plants and the portion of earth where the plant was growing, is mentioned. In 1914 a disease of betel-vines was reported from Bogra district of the Rajshahi division and was found to be doing considerable damage. Shaw [1915] who investigated the problem at the time showed that the disease was caused by *Rhizoctonia destruens* Tass. (= *Sclerotium rolfsii* Sacc.) and he further found that this fungus was responsible for a similar disease in the districts of Nasik, Belgaum and Dharwar in the Bombay Presidency. No preventive work of any consequence was done in the betel-vine gardens and interest faded as the disease subsided and the loss became normal. In 1923 the disease became noticeable in the Howrah district in Bengal and since that time it has persistently increased. It was later reported from Chittagong and as enquiries extended it became evident that the disease was causing considerable loss and was fairly widespread, the affected area extending from Hooghly to Chittagong and northwards as far as Rajshahi and embracing the whole of the deltaic regions of the Ganges, Brahmaputra and Padma rivers.

When the disease became manifest in an epidemic form, the vines in whole *borojes* were affected and died and in some villages the loss had become so great that the cultivation of betel-vines seems to have been almost abandoned. The circumstances are such that it is difficult to obtain accurate information about the actual extent of loss but in recent years it is believed to amount to many lakhs of rupees per annum. The intensity of the disease varies considerably from year to year. In 1927 when there was a light monsoon and practically no flooding the disease was almost entirely absent in the places where it was being investigated

and therefore under careful observation, while in the previous and succeeding years it was severe.

Foot-rot diseases of betel-vine seem to have a wide range in India, indeed, they have been reported from most of the major betel-vine growing areas. Reports of these diseases have been made by Dastur [1926] from the Central Provinces, by Sundararaman [1928] from the Madras Presidency, by U. Thet Su [1929] from Upper and Lower Burma, by Uppal [1931] from the Bombay Presidency and specimens have been received at Pusa from Chattarpur, Nagod and Bundelkhand States of the Central India Agency. Thompson [1929] reported it from Malaya and stated that the damage done was considerable.

SYMPTOMS OF THE DISEASE.

The earliest symptom of the disease is a darkening of the stem at the 'foot' of the plant near the ground level. The position of the darkened lesion may be from just below to about six inches above ground level or occasionally higher up. The leaves soon after begin to droop and become flaccid, the shoot wilts and in three or four days is withered and dead. Meantime the lesion on the stem has deepened and the discoloured area has extended upwards more rapidly than downwards but the extension is slow. Usually about three nodes and internodes become involved by the time the vine has died. The darkened part of the stem shrinks, becomes soft and sometimes slimy, and the colour ultimately becomes black. The epidermis may burst and the cortex rot exposing the fibres. The stem breaks very readily at the nodes within and just beyond the discoloured area. The lesion may extend from the upright stem along the part of the stem lying on the ground and the next shoot in its turn may wilt and die. It may also pass from one stem lying flat to the others of which there are often six to ten or more lying alongside it.

At an early stage if the wilting part of the shoot above the lesion is cut off and placed in water or in damp soil, the leaves become turgid as in the natural state and if the discoloured part and the adjacent internode from the healthy part of the plant are cut off, the rest of the plant grows healthily indicating that the disease is confined to the discoloured part of the stem. On it several fungi have been found among which *Sclerotium rolfsii* Sacc., *Rhizoctonia solani* Kuhn. and a Phytophthora have proved to be parasitic. A Colletotrichum and a Gloeosporium are very often present together with an ascomycete. Single spore cultures of the black form with setae, Colletotrichum, and of the pink from without setae, Gloeosporium, have produced each other and the ascigerous stage and *vice versâ*. Often in monosporous cultures on corn meal agar the pink form is produced first and the

black form later and cultures of each have produced asci sometimes within eight days. The acervuli are round or oblong, black or pink, with or without setae; conidiophores are 25-50μ; conidia hyaline or pink in mass, granular, rounded at the ends, 11-32 ×4-5·5μ; perithecia round 80-150μ in diameter; ostiolum papillate, short or long, 30-150μ, lighter coloured and sometimes hairy at the tip; asci 8-spored, biseriate; paraphyses absent; ascospores hyaline, oblong, or slightly fusoid, unicellular, rounded at both ends, granular, centre hyaline, 14-18×6-8μ. They put forth germ tubes and appressoria. Shear and Wood [1913] obtained a doubtful Glomerella associated with a Colletotrichum with numerous setae on the leaves of *Piper macrophyllum* Gray. They found the perfect stage in culture only but no mature spores were present. They included the species provisionally under *Glomerella cingulata* (Stoneman) Spauld and Schr. Stoneman [1898] described and figured most of her species as having perithecia with a long and distinct neck and created a new genus Gnomoniopsis. Dastur's work [1920] however has brought out clearly the variation of the perithecia in *Glomerella cingulata* in shape and size and in the presence or absence of setae in the conidial stage. All three forms of the fungus on betel-vine in Bengal and Assam are considered therefore to belong to *Glomerella cingulata*. Pure cultures of this fungus have not induced any disease in betel-vines and the experiments have been carried on for a sufficiently long time to lead to the belief that it is not the direct cause of disease, though, when it is placed on a young lesion caused by Phytophthora, it grows rapidly and masks the presence of the latter.

Other fungi are frequently found on the dead part of the stem such as species of Fusarium, Cephalosporium, Vermicularia, Aspergillus, Rhizopus and Phoma but all these seem to be saprophytic. Nematodes are often present in and on the soft rotten tissues but they cause no lesions and do not seem to be in sufficient numbers to lead to the supposition that they adversely affect the stem and no work has been done on them.

Causal organism and infection experiments.

Infection experiments with the fungi isolated from diseased betel-vines were begun in 1926. Small huts reproducing as closely as possible the *borojes* in the betel-vine growing area of Bengal were constructed behind the plant houses at Pusa. No betel-vine had been grown in this vicinity in recent years and the nearest *pan* gardens were about ten miles away. Soil from a severely infected *boroj* in Rishra near Chinsurah was brought to Pusa on the 8th of September. Part of it containing pieces of diseased stems was next day placed just below the surface of the soil around nine healthy plants. In another *boroj*, part of the same soil

without any pieces of stems, but having only organic debris, was incorporated on the 12th of September with the surface soil around six healthy plants. The record of deaths in each, in the order in which they took place, is given in Table I.

TABLE I.

Effect of soil from severely infected borojes *on healthy betel-vine plants. (A) soil with and (B) without fragments.*

Plant No.	Date of death	Fungus observed	Plant No.	Date of death	Fungus observed
	A			B	
1	13th Sept. 1926	Phytophthora	1	23rd Oct. 1926	Rhizoctonia
2	26th Oct. 1926	Phytophthora	2	26th Oct. 1926	Phytophthora
3	17th Nov. 1926	Rhizoctonia	3	26th Nov. 1926	Phytophthora
4	17th Nov. 1926	Rhizoctonia	4	10th Dec. 1926	Phytophthora
5	17th Nov. 1926	Phytophthora and Rhizoctonia	5	29th Jan. 1927	Rhizoctonia
6	10th Dec. 1926	Rhizoctonia	6	29th Jan. 1927	Rhizoctonia and Fusarium
7	6th Jan. 1927	Phytophthora			
8	1st Feb. 1927	Rhizoctonia			
9	30th Mar. 1927	Rhizoctonia			

Soil round fifteen other plants was moved in the same way but infected soil was not added in this case. These fifteen plants remained healthy to the very end. The Phytophthora and Rhizoctonia found destroying the plants were brought into culture. This experiment shows that both Phytophthora and Rhizoctonia were carried from Rishra to Pusa by means of soil with and without fragments of diseased stems and infected healthy plants.

Rhizoctonia sp.

The Rhizoctonia found on the 17th November was brought into culture and on the 30th of November small pieces of the culture medium with a good growth of the fungus were placed (*a*) in the soil near the plants, (*b*) on the part of the plant where it emerges from the soil and (*c*) on the same part of the stem which was slightly wounded before applying the culture. The record is as follows :—

TABLE II.

Infection experiments with Rhizoctonia sp. on betel-vines.

Plant (*a*)	Date of death	Fungus found	Plant (*b*)	Date of death	Fungus found	Plant (*c*)	Date of death	Fungus found
1	15th Feb. 1927	Rhizoctonia	1	14th Nov. 1926	Rhizoctonia	1	4th Dec. 1926	Rhizoctonia
2	11th Mar. 1927	Do.	2	8th Dec. 1926	Do.	2	10th Dec. 1926	Do.
3	21st Mar. 1927	Do.	3	6th Jan. 1927	Do.			

In each case that part of the stem near the ground level became discoloured and the shoot wilted from one to four days after the first sign of flaccidity became apparent. Portions of the stem were sectioned and incubated after surface sterilisation. The fungus was recovered from the discoloured portions only and not from the green and naturally brown portions beyond and microscopic examination of the fungus and its cultural characters established its identity with that used to inoculate the healthy plants. During the course of the experiments two other plants, that had not been infected with the fungus intentionally, died in the two huts, one on the 8th of February and the other on the 14th of April. The infected part of one of them was two feet above the ground and of the other just above ground level. Rhizoctonia was found in each. Neither of the plants was immediately close to the place where the infective material of Rhizoctonia was placed and it is likely that they were infected owing to handling during the operations of tending the plants. Similar experiments were repeated in this and succeeding seasons with confirmatory results showing that the Rhizoctonia isolated from diseased betel-vines is pathogenic. The presence of this Rhizoctonia as a cause of death of these vines in the experiment led to its being looked for in the field. In 1927 the disease was very light and this Rhizoctonia was found sparingly on diseased vines during the latter part of the monsoon and it gradually increased till November when it was found that it was the main cause of the death of the plants. During succeeding years Rhizoctonia was found sparingly during the hot weather and the monsoon, more abundantly during October and November and again sparingly throughout the cold weather. In a few places it was more prevalent than Phytophthora but at its worst never caused so many deaths as did the latter fungus. Mycelial threads are seldom so abundant under natural conditions as to be discernible by the naked eye, so there is in most cases little to

show whether this fungus or another is the cause of death without microscopic examination and cultural studies.

Identity of the Rhizoctonia.—The young hyphae branch freely and fusion between cells occurs frequently. The branch is constricted as it leaves the parent hypha and the septum is usually a little beyond the place of origin, from 3 to 33μ with a mean of 11μ. In a vigorously extending culture the branches come off at an acute angle and lie more or less parallel to the parent hypha but in older or in slowly growing cultures the angle is wider. Within the tissues of the stem of the betel-vine plant and also in young cultures the hyphae are colourless but they become yellowish brown and finally dark brown with age. The cells vary greatly in length measuring in a young vigorously growing culture 36-342 × 6-10·5μ with a mean, based on two hundred measurements, of 118 × 7·8μ, half the measurements lying between 80 and 160μ. In old cultures, however, while the length of the hyphae is less the breadth may reach 12μ. Sclerotia form slowly in culture and their development is typical. They are irregular in size and shape; some are globular, others are flat and elongated. They may reach 3 mm. in diameter. The surface is covered with light brown fluffy hyphae that may extend to one millimeter. The tissue within is compact, dark brown, and in the larger specimens more or less distinctly zonated. Several sclerotia may coalesce to form a stromatic mass which may reach 1·5 centimeters in breadth and sometimes small drops of a light to dark brown liquid are exuded on the surface.

For the measurements of the cells of the hyphae of *Rhizoctonia solani*, Duggar [1916] gives 100-200 × 8-12μ and according to Peltier [1916] 65·24 × 5·01-6·57μ, though he states that the length of the cells of the strain parasitic on a species of Dianthus is 50-125μ. Palo [1926] gives for the strain parasitic on rice 24-248 × 4-12·8μ and Bourn and Jenkins [1928] give 50-525 × 5·5-12μ for the strain parasitic on aquatic plants. The measurements of the strain parasitic on betel-vine are a little smaller than those given by Duggar and close to those given by Palo and Bourn and Jenkins. It is safe therefore to consider this fungus as a strain of *Rhizoctonia solani* Kuhn.

Sclerotium rolfsii Sacc.

In March 1927 a wilting vine was observed at Dacca whose stem at ground level was discoloured and was almost covered with a white mycelium. This vine was brought to the laboratory and kept under observation and in a few days' time a few brown sclerotia were produced on the surface. Cultures were then made and the fungus was identified as *Sclerotium rolfsii* Sacc. In order to see if the fungus was pathogenic, infection experiments were started on the 1st of May 1927. Five healthy betel-vine plants were selected and a sclerotium was placed at ground level

near each one of the plants. Next day hyphae from the sclerotium were growing on four of them. On the succeeding day the mycelium was fairly copious and the stem under it was becoming brown. On the fifth day the surface of the stem for a length of from three to five centimeters from the place of inoculation was brown, soft and rotten and easily broken away at the nodes near the infected place. The white mycelium was running along the stem in straight lines and radiating in a white fan-shaped weft on the surface of the soil to a distance of five centimeters. Sclerotia were forming as small white pin-heads on the mycelium, both on the stem and soil. The upper part of the vine showed signs of wilting on the third day and on the fifth day it had collapsed. The bulk of the mycelium remained outside the stem but hyphae were traced in and among the cells of all the tissues, where they broke down the middle lamella so that the cells fell apart causing a soft rot. The sclerotium that was placed on the fifth vine did not produce hyphae and when it was transferred to the culture medium, did not do so either. Twenty sclerotia from the original culture tube had produced the mycelium within twenty-four hours, so it is likely that this sclerotium was killed by too hot a wire as it was being placed in position on the plant. Another sclerotium placed on this same plant developed mycelium and the plant, showing signs of wilting on the third, collapsed on the fifth day. Five other plants under the same conditions but without the fungus remained healthy. Hyphae were found within the tissues both between and in the cells. From such internal hyphae cultures were obtained after strong surface sterilisation with mercuric chloride solution and flaming and these were like the original culture of *Sclerotium rolfsii*. These cultures were as virulent as the original and infected five vines in four to five days reproducing the exact symptoms as before. The mycelium radiated on the soil in a loose cottony weft as far as ten centimeters from one of the decaying stems and grew on fallen leaves. Sclerotia developed copiously on this mycelium and were brown and mature in ten days. Placed in nutrient agar, they developed the mycelium within twenty-four hours and placed on four healthy vines, they caused them to wilt in four days. A piece of discoloured stem from a wilted plant attacked by *S. rolfsii* was placed at ground level in contact with a healthy shoot and the latter began to wilt in three days. Hyphae were seen in the tissues of the discoloured portion of the stem of the wilting shoot and from it the fungus was also recovered. Similarly from another piece of discoloured stem placed on the soil five centimeters from a healthy shoot, mycelium grew on the soil surface, reached the shoot in three days and the plant wilted in four days. Four cuttings placed in the soil at places from which four wilted plants had been removed became infected and died within five days. Cuttings placed in exactly similar positions but without contact with infected soil or plants struck roots and grew vigorously. These experiments

showed that dead infected parts of the stem were a potential danger to vine growing and that the disease was carried by the soil also.

From the pot experiments conducted in sterilised soil under very humid conditions, it became apparent that a high degree of humidity is necessary for the vigorous growth and successful infection of betel-vines by *S. rolfsii*. These observations are in accord with the experience of Rolfs [1896], the original investigator of this fungus, of Peltier [1916] who worked with perennial garden plants in Illinois and of Bertus [1927] who worked with groundnuts in Ceylon. A high temperature seemed also to be conducive to disease production and between 32° and 35° the fungus was most virulent, infecting every plant with which it came into contact. In these conditions however the Phytophthora and the Rhizoctonia failed to parasitise the host.

Identity of the Sclerotium :—As already stated this fungus grew luxuriantly with copious formation of sclerotia on Quaker Oats agar. On other common media such as corn-meal agar, potato dextrose agar, Richards' agar, it grew also, exhibiting a preference for those media that were rich in composition. The young vigorously growing mycelium is white and somewhat lustrous. The hyphae are hyaline, vacuolated, and measure from 3 to 8μ in diameter. Old hyphae contain oil drops and numerous granules and the mycelial mass at this time loses its lustre. Clamp connections are common. Hyphae often run parallel to one another with frequent anastomosis and form loose strands. Sclerotia occur singly or occasionally joined together, are spherical, oblate or slightly elongated, brown, shiny, and smooth at first but become wrinkled and dull with age and on drying. They measure 0·8 to 2 mm. in diameter. Smith [1899] gives ·8-·9×1·2-1·3 mm., Saccardo [1911] ·5 to ·8 mm., Peltier [1916] ·8 to 3 mm., Bertus [1927] 1 to 8 mm. on various media, and Rhind [1926] mentions two distinct strains on groundnut, one with large dark brown sclerotia and the other with smaller paler ones. Thus the size of sclerotia varies considerably in different conditions or in different strains. Those found on the stem of the betel-vine and the culture on Quaker Oats agar are similar in size while those on potato slabs are slightly larger. The sclerotium is parenchymatous, with outer cell layers made up of more or less flattened but thick brown walls. The cells of the layer immediately within are slightly tinted while those farther inside are thin walled and hyaline and more loosely packed towards the centre. The fungus agrees therefore with the published descriptions of *Sclerotium rolfsii* Sacc.

Phytophthora spp.

A number of wilting betel-vine plants were also brought on the 8th of September 1926 from Banhoogli near Calcutta and Santragachi near Howrah, which showed

the presence of a phycomycetous fungus. Mycelium and sporangia were few in number but pieces of stem kept in a little water in a moist chamber overnight produced sporangia in fair abundance. Pure cultures were made in the usual way, (*a*) by plating sporangia from the stem and (*b*) from a piece of the stem with the rind pared off and washed in 0·1 per cent. corrosive sublimate, then in sterile water. French bean agar was used for cultivating the fungus.

Fragments of culture medium with a copious growth of mycelium were kept overnight in a moist chamber. They were then wetted and when the sporangia began to discharge zoospores a fragment was placed on each of the six healthy plants. The position varied from just below ground-level to four inches above this. Some of the plants were slightly wounded by scraping off the epidermis at the spot where the infective material was to be placed and such plants are indicated by an asterisk in Table III where the results are recorded. A minute piece of cotton wool was placed over the infective material and fixed in position.

TABLE III.

Parasitism of Phytophthora sp. isolated from betel-vines.

Plant No.	Date of inoculation	Date of first symptoms	Date of wilting
1*	16th September 1926	19th September 1926	20th September 1926.
2*	,, ,, ,,	,, ,, ,,	,, ,, ,,
3	,, ,, ,,	,, ,, ,,	,, ,, ,,
4	,, ,, ,,	22nd ,, ,,	23rd ,, ,,
5	,, ,, ,,	,, ,, ,,	,, ,, ,,
6	,, ,, ,,	Remained healthy till the 17th November 1926 when the experiment was terminated.	

The fungus used in this infection experiment was isolated by the method designated as (*a*) above. Adjacent plants that were treated as controls did not become infected. The symptoms were the same in the wilting and dead plants as already described. In the next experiment the results of which are recorded in Table IV, the fungus used was secured by the method designated as (*b*).

TABLE IV.

Parasitism of Phytophthora sp. isolated from betel-vines.

Plant No.	Date of inoculation	Date of first symptoms	Date of wilting
1	20th September 1926 .	24th September 1926 .	25th September 1926.
2	,, ,, ,, .	23rd ,, ,, .	,, ,, ,,
3	,, ,, ,, .	24th ,, ,, .	,, ,, ,,
4	,, ,, ,, .	,, ,, ,, .	,, ,, ,,
5	,, ,, ,, .	23rd ,, ,, .	,, ,, ,,
6	,, ,, ,, .	11th November ,, .	21st November ,,

Six adjacent plants treated as controls remained quite healthy. The wilting vines showed the usual symptoms such as discoloration of the 'foot' and the symptoms associated with wilting. From nine of the plants in the two experiments a Phytophthora was recovered which agreed in mycelial, sporangial and resting spore characters with the original culture of Phytophthora. An infected plant in each experiment was left in position for some time. The tissue of the discoloured part rotted away into a soft pulp exposing the fibres and the stem at the nodes near the rotton portion could be broken with the slightest pressure. Microscopic examination showed the presence of non-septate hyphae indicating the presence of the Phytophthora in the tissues.

The infective material was procured from plant No. 5 of the last experiment by plating sporangia and with this material yet another test was done. The data are recorded in Table V.

TABLE V.

Parasitism of Phytophthora sp. from plant No. 5 of the previous experiment.

Plant No.	Date of inoculation	Date of first symptoms	Date of wilting
1	7th October 1926 .	11th October 1926 .	12th October 1926.
2	,, ,, ,, .	,, ,, ,, .	,, ,, ,,
3	,, ,, ,, .	12th ,, ,, .	13th ,, ,,
4	,, ,, ,, .	negative . . .	....
5	,, ,, ,, .	negative . . .	....
6	,, ,, ,, .	negative . . .	....

Six plants that were treated as controls along with these plants remained healthy all the time. Three of the inoculated plants did not somehow become infected but why they did not is not known. It does not seem to be that they were resistant because in tests carried out later it was found that they became infected on the second trial in conditions that did not seem to differ in any way from those of the first trial. In the other plants the darkening of the stem at the point where infective material was placed, the spread of the discoloration in the stem and then wilting of the shoot were like the symptoms of the diseased plants in the field. From the infected portions a Phytophthora was recovered which was identical with the original one in all respects.

In these three experiments the plants were few as the total number available in Pusa then was small. The sequence proving that this Phytophthora is parasitic on the betel-vine, and that it causes rotting in the lower part of the stem and wilting of the shoot is, however, complete. Infection experiments were repeated many times in succeeding years when more betel-vines became available but since the results are confirmatory they need hardly be given in detail. Successful infection occurred only when conditions were very moist. In Pusa where the humidity is often 80 in the early morning but much less during the day, betel-vines could not be infected in the open air but only when the part of the plant on which the infective material was placed was either held in a glass funnel in which the air was kept moist or covered by a little cotton wool that was kept continuously wet. Similarly in the laboratory infection took place when the plants growing in pots were kept in conditions of high humidity. In the cold weather with temperatures ranging from 10° minimum to 26·5°C. maximum, infection was rare but in the hot weather and the monsoon with temperatures ranging from 24° minimum to 35°C. maximum, infection experiments were invariably successful.

The young mycelium is unseptate but, as it grows older, septa appear as is usual in the genus especially as the cell contents are withdrawn and though sparingly present they are greater in number than has been usually the case with the species examined in this laboratory. Aerial hyphae are long, sparingly branched, and uniform in diameter while submerged hyphae are shorter, much branched, have often numerous protuberances and are irregular in diameter. They are from 3 to 7μ in diameter, the average being 5μ. The contents are hyaline and coarsely granular. Sporangia are borne on aerial hyphae and are terminal, lateral or intercalary. On the betel-vines, sporangia are few. On the culture media they are also few till about the fifth day but are numerous on the tenth. When however the mycelium is submerged in water for a night, they develop copiously. Even a two days' old culture with no sporangia will produce them in considerable numbers when submerged. Typically the sporangium is symmetrical and pyriform with a prominent

papilla and a long conidiophore at the broad end but there is however great variation under different conditions. Growing in water the sporangia may be spherical or obovate and are often asymmetrical with the papilla more or less to one side and sometimes inconspicuous. On agar media, when about twenty days old or more, sporangia are often irregular, asymmetrical, obovate, or oblong or bent. Occasionally the upper part is elongated to form a sort of neck. Two papillae may be present and the conidiophore may be inserted on the side. When sporangia become free, in the majority of cases no part of the conidiophore adheres but occasionally the conidiophore remains attached like a long slender stalk. A large number of sporangia from maize meal agar, Quaker Oats agar, from the naturally infected stems, petioles and leaves of betel-vines were measured. The mean length and breadth and the range are recorded in Table VI.

TABLE VI.

Measurements of sporangia of Phytophthora sp. from betel-vines.

Medium	No. measured	Mean	Range
		μ	μ
Maize-meal agar to water*. . .	900	42·8×24·3	22-76×12-42
Do. 10 days old. 20° . .	200	54 ×32	22-80×24-43
Do. do. 30° . .	200	59·5×34	30-132×22-48
Quaker Oats agar to water* . .	700	39·2×24·8	19-58×15-35
Do. 10 days old at 20°C . .	200	50 ×30	31-77×21-41
Do. do. at 30°C . .	200	59·6×32·4	33-114×22-42
Sporangia from stem . . .	100	40·0×20	24-56×15-25
Sporangia from petiole . . .	100	34 ×21·5	18-50×12-33
Sporangia from leaves . . .	100	38·4×26·3	25-55×17-33

* Indicates that the measurements were made at laboratory temperatures varying from 18 to 25°C.

It will be noted that the mean is somewhat different on different media and it is greater at higher temperatures. Sporangia obtained from the cultures are broader than those found on the stem. The sporangia germinate by the liberation of 16 to 20 biciliate zoospores measuring 9-15×7-14μ when at rest, the average being 12×11μ. They settle soon and germinate quickly. Many sporangia especially the

larger ones germinate as conidia by producing germ tubes from any part of the surface, though usually from the papillate end. One or more germ-tubes may be produced from which secondary sporangia arise or they may develop into a branched mycelium.

Chlamydospores are few on the betel-vine and also in cultures till they are about fifteen days old after which they become fairly numerous. In water however they develop sooner and more copiously. They are subspherical with walls slightly thicker than those of the sporangia and slightly coloured yellow when old. In water they measure 20 to 49μ, the average being $29\times28\mu$. In oat-agar they measure from $20\text{-}50\times20\text{-}48\mu$, the average being $34\times32\mu$. In old cultures they have been found germinating and when kept moist from 5 to 10 days, they produce branched hyphae.

Oogonia have developed sparingly in three-month old cultures on Quaker Oats agar, maize-meal agar and green pea agar. They are deep brown, slightly oblate, with walls from 2 to 4μ thick, the average being 3μ. The oospores are free in the oogonia, slightly lighter brown, spherical, with walls 1 to 4μ thick, the average being 2μ. The thickness of the wall is slightly irregular and the surface smooth or slightly unevenly corrugated. The antheridium is amphigynous and thin-walled. The measurements of these organs are given in Table VII.

TABLE VII.

Measurements of oogonia, antheridia and oospores.

Media	No.	Oogonia		Antheridia		Oospores	
		Range	Mean	Range	Mean	Range	Mean
		μ	μ	μ	μ	μ	μ
Quaker Oats . .	74	20—52	38	7×20	$11\cdot5\times13\cdot5$	14—40	30·5
Maize-meal . .	75	27—44	35	8×18	$12\cdot0\times14\cdot0$	19—36	28·0

It may be mentioned that the oospores have not been found in the tissues of the diseased plants in the field but it is not unlikely that they do occur, for, as has been mentioned in the beginning of this paper, the opportunity for observation is definitely restricted.

Indentity of the Phytophthora spp.—By comparing the data on the morphology spore measurements and temperature relations (to be discussed hereafter) with those of species of Phytophthora investigated by others, it should usually be

possible to determine the specific rank of the fungus. But the Phytophthoras are unfortunately weak parasites, in some cases at least, without much host specialisation. They are furthermore highly plastic, the size of the sporangia being especially susceptible to wide variation with the variation in composition of the culture media and temperatures. Several attempts have been made by investigators to gather and grow all the available species under identical conditions and then place the taxonomy of the species on sound lines. The earliest attempt was made by Rosenbaum [1917] who brought a dozen species together, noted the size and morphology of the sexual and asexual reproductive bodies, the shape of the papilla, variation in mycelium and other characters and thus tried to separate the genus into different groups. He was the first mycologist to introduce the concept of analysing the sporangial measurement data using biometrical methods in order to study the variation which exists in the measurements of length and breadth. He measured as many as 400 sporangia of each species under study with a view to get a representative sample from the general population.

Leonian [1925], dissatisfied with the taxonomy of the group based on the size and shape of the reproductive organs and the nature of the serial and submerged mycelium, tried to base a key on physiological reactions. He discarded the morphological differences as being too variable. His exhaustive study included a large number of known and unknown species but it has not, however, solved the problem of the taxonomy of this genus as it relies too much on physiological responses for the separation of the species.

Dufrenôy [1926] considered that there were sufficient differences in the chemical, composition of the protoplasm to determine the specific rank. Gadd [1927] considered the morphology and size of the sexual organs to be equally reliable. The most recent treatment is that of Tucker [1931] who studied 150 isolates. He took as the most reliable characters for the differentiation of the species the ability to grow on certain media, type of antheridium, character of the sporangia, temperature toleration and in a few species the development of certain type of reproductive organs, size of oospores and pathogenicity. Biometric analysis of the size of the fruit bodies alone was not considered sufficient but was considered along with other characters that were also studied and to which equal importance was given.

In order to arrive at a decision regarding the taxonomic position of the betel-vine Phytophthora the following studies were undertaken :—

1. Rate of growth at three high temperatures.
2. Pathogenicity.
3. Measurements of sporangia and their statistical analysis.

The following isolates from betel-vines and known species were included in this study :—

Phytophthora from betel-vine, Bengal strain.

Phytophthora from betel-vine, Madras strain. Sent by Mr. S. Sundararaman.

Phytophthora from betel-vine, Malaya strain. Sent by Mr. A. Thompson.

Phytophthora parasitica Dastur from *Ricinus communis* L. from Pusa.

Phytophthora palmivora Butler from *Borassus flabelliformis* from Godaveri, from Mr. S. Sundararaman.

Phytophthora meadii McRae from *Hevea brasiliensis* from Cochin, from Mr. S. Sundararaman.

Phytophthora colocasiæ Rac. from *Colocasia antiquorum* Schott, Pusa.

Strains of Phytophthora causing the foot rot of betel-vines in the Bombay Presidency sent by Uppal and in Bundelkand were received too late for inclusion in the spore measurement studies but were included in the temperature toleration studies.

1. Rate of growth at three high temperatures.—The isolates were grown on maize meal agar made according to the method of Shear and Wood [1913]. Twelve cubic centimeters of the sterile medium were poured into Petri dishes of 100 mm. diameter of which there were ten for each temperature. The cultures used in inoculating these plates were grown for two generations on corn meal agar and incubated at 24°C. Only three temperatures were tried and incubators were accurately set to the desired temperatures using newly standardised thermometers. The growth of the colonies was measured daily and the results obtained are recorded in Table VIII.

TABLE VIII.

Linear rate of growth of some Phytophthoras at three different temperatures

Strain	32°C.			35°C.			37°C.
	2nd day	3rd day	4th day	3rd day	4th day	5th day	—
Bengal	68·5	80·0	95·0	34·5	42·5	47·2	..
Madras	11·5	21·2	35·0	3·0	3·5	3·5	..
Malaya	10·2	21·2	36·7	10·2	10·2	10·2	..
Bombay	40·5	56·0	72·5	5·0	5·0	6·7	..
Bundelkand	44·0	61·1	80·6	7·7	8·7	8·7	..
P. parasitica	54·5	78·0	95·0	14·6	15·0	16·0	..
P. palmivora	44·7	66·2	95·0	5·0	6·2	6·2	..
P. meadii	39·0	53·3	75·0	..	..	..	..

All strains failed to grow at 37° while *P. meadii* did not grow even at 35° which distinguishes it from the others. Aside from this, other relationships are not evident from this study.

2. Pathogenicity tests.—Infection experiments with the various isolates were done on *Colocasia antiquorum*, *Ricinus communis* and *Piper betle*.

Colocasia antiquorum.—All the strains and species could penetrate and cause brown spots on the leaves of this plant. Those due to *P. colocasiæ* were always ahead of the others in size, the largest being 70×60 mm. on the tenth day and concentraic rings were conspicuous and drops of a pale yellow liquid were always present. The spots caused by other Phytophthoras were never concentric and never had drops of any liquid. The average diameter of the spots due to each fungus is given in Table IX.

TABLE IX

Diameter of the spots caused by Phytophthora spp. on Colocasia antiquorum

Size	Bengal	Madras	Malaya	*P. parasitica*	*P. meadii*	*P. colocasiæ*
	mm.	mm.	mm.	mm.	mm.	mm.
Maximum . .	14×8	10×4	28×20	18×15	25×20	70×60
Minimum . .	8×4	8×4	14×9	9×5	16×9	34×27

By its reaction on this host *P. colocasia* can be distinguished from the other species and strains. It becomes evident also from the nature of the spot produced and its size that the betel-vine strains are distinct from *P. colocasiæ*.

Ricinus communis.—These plants were about six inches high at the time of inoculation with several leaves. Thirty-two plants were available for infection work. On applying the inoculum, the spots appeared on the second day and extended in size until the leaves drooped and withered and the infection spread quickly to the stem. While those infected with *P. colocasiæ* did not take infection, all the others did. Ability of the betel-vine Phytophthora to infect the castor plants and the inability of *P. colocasiæ* to do so is a further proof that they are not identical.

Piper betel.—All the strains of Phytophthora from betel-vines produced typical lesions but all the others failed to infect this host. These tests were repeatedly made and every time the same results were obtained.

A consolidated table giving the results of the tests carried on the three hosts with the several fungi is given below.

TABLE X.

Infection experiments with phytophthoras on Colocasia antiquorum, Ricinus communis *and* Piper betle.

Host	*Colocasia antiquorum*													*Ricinus communis*											*Piper betle*					
Year	1930					1931					1932					1931				1932						1932					
Date of inoculation	22/7	8/8	15/8	13/9	11/12	4/6	15/6	4/7	14/7	17/8	14/7	4/8	22/9	28/9		17/7	28/7	19/8	24/9	1/7	8/7	12/7	22/7	15/8	15/9	26/7	26/7	11/8	27/9	9/11	23/12
Temp. mean max.	33	33	34	31	21	43	37	32	35	35	32	33	34	31		32	33	31	34	33	34	34	32	32	33	33	32	34	34	28	24
Temp. mean min.	26	26	24	25	17	28	26	25	26	27	26	26	25	24		26	26	27	25	26	26	26	26	25	26	26	26	25	25	16	10
No. of plants infected per strain.	15	12	15	12	8	6	12	6	6	6	8	8	6	6		8	10	9	8	10	10	8	8	8	6	6	10	8	8	5	6
Bengal	0		10	1	0	5	2	3	3	3	5	2	4	4		6	6	9	8	8	8	7	8	5	5	6	6	1	1	2	1
Madras	0	8	8	0	..	..	..	2	3	2	4	3	2	4		5	3	8	8	8	8	5	6	4	3	2	3	0	0	0	1
Malaya	0	5	10	0	..	..	4	0	3	4	2	5	3	3		4	5	7	6	8	6	7	5	5	2	1	2	0	0	1	0
P. meadii	13	7	8	..	..	4	1	1	3	2	6	4	2	2		4	4	8	7	8	8	1	2	6	4	0	0	0	0	0	0
P. parasitica	..	..	..	..	..	..	4	8	6	5	2	5	3	3		6	8	9	8	8	9	7	8	8	5	0	0	0	0	0	0
P. palmivora	..	..	..	..	..	..	..	..	..	..	3	3	5	2		..	..	..	..	7	3	6	3	5	3	0	0	0	0	0	0
P. faberi	..	..	..	..	..	..	..	..	..	..	2	2	4	2		..	..	..	..	7	4	3	2	6	3	0	0	0	0	0	0
P. colocasiae	15	8	10	8	0	1	2	6	6	6	6	8	6	5		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Measurements of sporangia.—The isolates were grown for ten generations on Quaker Oats agar before the study regarding sporangial measurements was undertaken. Finally in order to obtain sporangia that were of the same age and were grown in indentical conditions, the agar medium was made in one batch, poured into test-tubes, and after inoculating with the isolates, the tubes were incubated for four days at 20°C. On the fourth day all the cultures had formed profuse mycelium with the exception of the Malaya strain which was rather tardy in growth. After making sure that the mycelium was free from sporangia, fragments were transferred, twenty-four hours before starting the measurements, to watch glasses containing sterile water and placed in the incubator. The next morning the watch glasses were placed in bright light near a north window. By following this method originally suggested by Klebs, abundant sporangia that had freshly formed were made available for measurements. The length and breadth of four hundred sporangia were measured from each strain and species, and the measurements were recorded in adjacent columns.

Rosenbaum [1917] and others who have used statistical methods for comparing the spore measurements determined the significance of mean differences in length and breadth existing between different known and unknown species by dividing those differences by the probable errors of the respective mean differences. When however more than two cultures are under study this method is beset with certain difficulties and the sporangial measurements as a taxonomic criterion are then not of much value. Mahalanobis [1933] has suggested a new coefficient, the coefficient of divergence (delta^2) for the comparative study of anthropometric data and it was thought that that coefficient would be of value in arriving at correct conclusions. The data of sporangial measurements were sent to him therefore to obtain his opinion regarding the relationships between the unknown forms of betel-vines and the known species, based on the application of his coefficient. The biometric constants obtained by him from these data are recorded in Tables XI and XII.

TABLE XI.

Mean standard deviation and coefficient of correlation (' r ') *of 400 sporangia from each of three unknown strains of Phytophthora and five known ones.*

Species or strain	n	MEAN		STANDARD DEVIATION		"r"	Probable error of 'r'
		Length	Breadth	Length	Breadth		
Bengal . .	400	40·03±·28	27·62±·19	8·22±·19	5·74±·14	·736	±·015
Madras . .	400	34·62±·26	21·38±·12	7·68±·18	3·70±·09	·473	±·026
Malaya .	400	41·75±·30	27·00±·16	8·85±·21	4·81±·12	·552	±·023
P. parasitica .	400	40·50±·26	27·04±·14	7·65±·18	4·67±·12	·602	±·021
P. meadii . .	400	31·64±·24	22·04±·14	7·15±·17	4·18±·10	·836	±·011
P. colocasiae .	400	43·23±·38	21·76±·17	11·20±·27	4·52±·11	·787	±·013
P. faberi . .	400	36·73±·23	27·56±·15	6·85±·16	5·07±·12	·825	±·015
P. palmivora .	400	39·60±·28	25·42±·16	8·33±·20	4·83±·12	·738	±·015

TABLE XII.

Δ^2, the generalised distance between the mean values of the various known and unknown species of Phytophthora.

	Bengal	Madras	Malaya	*P. parasitica*	*P. meadii*	*P. colocasiae*	*P. faberi*	*P. palmivora*
Bengal . .	..	·4723	·0377	·0127	·0223	·2441	·1231	·0787
Madras . .	·4723	..	·4600	·5180	·1448	·3527	·7967	·2351
Malaya . .	·0377	·4600	..	·0103	·5308	·7513	·2810	·0288
P. parasitica .	·0127	·5180	·0103	..	·5053	·1203	·2234	·0399
P. meadii .	·0223	·1448	·5308	·5053	..	·3458	·4285	·2862
P. colocasiae .	·2441	·3527	·7513	·1203	·3458	..	·6336	·7408
P. faberi . .	·1231	·7967	·2810	·2234	·4245	·6336	..	·3885
P. palmivora .	·0787	·2351	·0288	·0399	·2862	·7408	·3885	..

From the data recorded in Tables XI and XII, Professor Mahalanobis drew very interesting conclusions. Regarding the nature of the frequency distributions for length and breadth of the sporangia of the eight isolates under study, he considered them as being roughly normal even though *P. meadii* shows slightly significant differences and the Bengal and Malaya strains show certain abnormalities suggesting heterogeniety or instability, to which however he did not attach much importance unless these indications were confirmed on larger samples. Confining the attention to the size of the sporangia and calculating the generalised statistical distance between each pair of samples, he states in a letter :

"The main results are shown in a diagrammatic form in the accompanying chart (Fig. 1). In this diagram each sample is represented by an ellipse with its centre on a point showing the mean length and mean width. The shape and orientation of the ellipse is determined by the standard deviations of length and breadth and the coefficient of correlation."

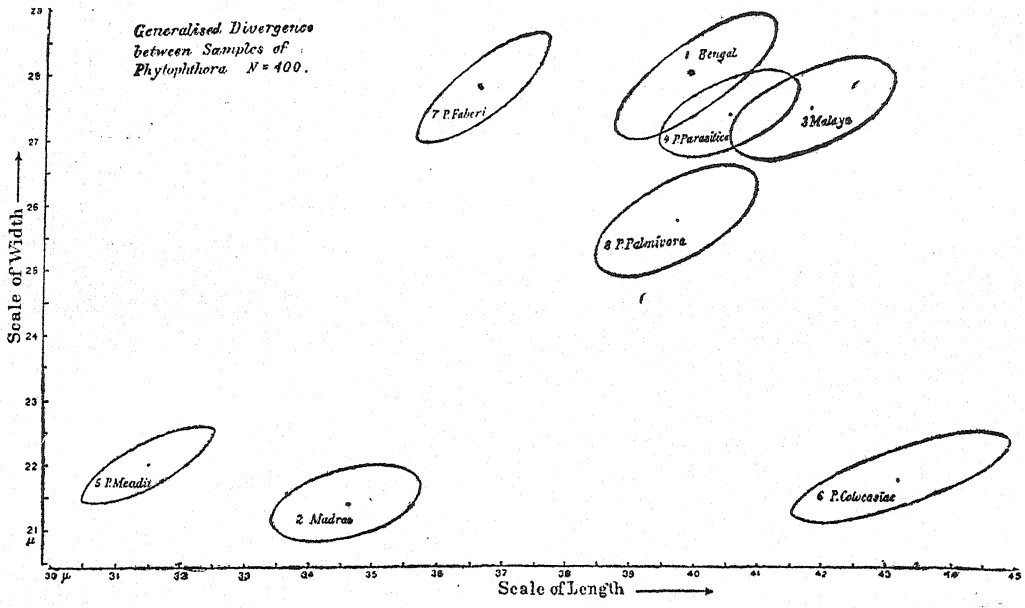


Fig. 1.

"Now if two ellipses coincide or nearly overlap it is clear that respective mean values for the two samples also coincide (or will be statistically indistinguishable) in most cases. The two samples may in this case be considered to have been drawn from the same population. On the other hand, when the two ellipses do not touch, it is clear that the probability of the two sets of mean values being in agreement will be less than one per cent. The greater the separation of the two ellipses the lower will be the probability of the mean values being in agreement. The separation of the ellipses then gives a measure of the generalised statistical distance between respective samples. It will be noticed that the coefficients in Table XII correspond to the separation between the ellipses." The chief results he has deduced are given below :—

1. Samples of *P. meadii*, *P. colocasiæ*, *P. faberi* and the Madras strain are all very clearly differentiated from one another and also from the remaining samples.
2. The sample of *P. palmivora* is also significantly differentiated from all other samples but in point of size shows a certain amount of resemblance to *P. parasitica*, the Bengal and Malaya strains.
3. The ellipses for samples of the Bengal and *P. parasitica* strains are overlapping to a certain extent showing close resemblance in size between these two.

4. The sample of *P. parasitica* is probably even more closely associated with the Malaya strain.
5. The Bengal strain is however clearly differentiated from the Malaya strain. Taking results stated in 3, 4 and 5 together, it appears probable that both Bengal and Malaya are connected with a more fundamental form, *P. parasitica*, but have undergone variations in different directions.
6. In many cases the samples show significant differentiation in standard deviations and coefficients of correlation which indicates that the factors producing variability and organic correlation between length and breadth are not identical for all samples.

It is thus evident that the Malaya strain and the Bengal strains, in their morphology, physiological responses and pathogenicity and their sporangial measurements resemble *Phytophthora parasitica* Dastur and are accordingly placed in that species. The description of Ashby's [1928] emended *P. parasitica* Dastur fits the Bengal strain except that the mean ratio of length to width of sporangia is sometimes greater than 1·4 and the oospores are mostly free in the oogonial cavity. Four hundred sporangia of this strain from a four-day old culture at 25°C. on oat agar, submerged in water overnight, had a mean L/W ratio of 1·45 and a mode of 1·4. The ratio L/W calculated from the figures in Table VI, are approximately 1·7 for sporangia from maize meal agar, 1·5 from Quaker Oats agar and 2·0 from the stem of *Piper betle*. Rosenbaum's [1917] work on Dastur's *P. parasitica* from castor shows that the predominant ratio L/W approaches 1·82 and the mean ratio is above 1·5 but Ashby thinks that it was evidently another species. The mean ratio would seem not to be constant but to vary with the substrata on which the fungus is growing so that a mean ratio of 1·4 or less is not a fixed point for this species. On the whole then the Bengal strain is considered as belonging to the Macrospora group of *P. parasitica*. It differs from *P. parasitica* isolated by Dastur from betel-vines in the Central Provinces because he states that the oospores in culture are similar in size to those of his strains of *P. parasitica* from castor and vinca which would put it in the microspora group.

The question of the taxonomic position of the Madras strain now remains to be determined. In its gross physiological and parasitical properties, this isolate shows affinities to *P. parasitica* but its sporangial measurements place it apart from known species that were included in this study. To consider it, in the face of this wide divergence, as a variety of *P. parasitica* or as a physiologic form of it would be stretching the meaning of that term too far. Physiological forms according to Stakman [1929] may be recognised by their parasitism, cultural characters, physio-

logical reactions and to a limited extent morphology. An isolate which differs so decidedly as the Madras strain does in its sporangial size from *P. parasitica* cannot be considered as a physiological form. The determination of the specific position of this Phytophthora is left for future work.

Thompson [1929] found that the Malaya strain of the betel-vine Phytophthora formed oospores in the Imperial Mycological Bureau at Kew which enabled Dr. S. F. Ashby to determine this fungus as *P. colocasiæ*. Reasons for placing this in that species are not specifically given and it is not clear whether a comparative study was done. Furthermore only the range and the mean of the sporangia, chlamydospores and oospores of this strain are given. He states that the Malaya strain did not parasitize *Colocasia antiquorum* and it did not form oospores under Malaya conditions while a type species of *P. colocasiæ* from America did. The sporangia of *P. colocasiæ* are stalked while ninety-eight per cent. of sporangia of the Malaya strain sent by Thompson to the author had no stalks. This particular isolate at any rate belongs to *P. parasitica*.

Attention may now be drawn in passing to the taxonomic position of *P. faberi* and *P. meadii* which Tucker [1931] has relegated to the position of synonymy of *P. palmivora*. The sporangial measurements of these species and their statistical analysis definitely rules out this suggestion and in the chart prepared by Professor Mahalanobis the statistical distance between these is wide.

TEMPERATURE RELATIONS OF THE THREE 'FOOT-ROT' FUNGI AND THEIR RELATION TO ENVIRONMENT

It has been stated before that in Bengal foot-rot due to *Rhizoctonia solani* appeared in a severe form only in the months of October and November while the ravages due to *Phytophthora parasitica* were confined to the monsoon months. Moisture and warmth seemed also to favour the production of the disease by *S. rolfsii*. These observations led to studies to determine the temperature relations of these fungi and then try *to correlate* the data so obtained with the periodicity of their appearance in the fields. All the fungi were grown on Quaker Oats agar for this purpose in Petri dishes that were of 90 mm. diameter. These dishes were carefully selected and were sterilised with proper precautions. Equal quantities of agar were poured in each and care was also taken to see that the depth of the medium was uniform in all. The inocula were placed in the centre of the poured plates twenty-four hours after pouriang, and the daily rate of growth was measured by determining three equiangled diameters. There were three plates for each temperature and each fungus and each diameter recorded in the tables below is therefore an average of nine readings.

TABLE XIII.

Growth in mm. of R. solani *from betel-vine at different temperatures in °C.*

Days	0°	10°	12°	17°	20°	24°	28°	32°	35°	37°	41°
1	0	0	0	3	17	16	19	16	11	0	0
2	0	0	0	15	57	55	58	31	23	0	0
3	0	0	0	39	89	89	87	44	37	0	0
4	0	0	0	61	..	..	..	62	54	0	0
5	0	0	4	86	..	..	..	89	68	0	0
6	0	0	7	..	..	..	..	..	74	0	0
7	0	0	9	..	..	..	..	..	82	0·6	0
8	0	0	10	(Growth here covered the entire					..	0·6	0
9	0	0	12	Petri dish)					..	0·6	0
10	0	0	14	..	..	..	..	..	..	0·6	0
11	0	0	17	..	..	..	..	..	..	0·6	0
12	0	0	20	..	..	..	..	..	..	0·6	0

At favourable temperatures which are from 20° to 28°, the mycelium attained a maximum diameter of 89 mm. in three days. Temperatures of 10° and below did not permit growth of the fungus and at the other end, 37° just permitted some growth. Dishes placed in the incubator at 41° were, after the twelfth day, placed on the laboratory table. The fungus, however, failed to grow indicating that prolonged exposure to that temperature had killed it.

The results obtained with *Sclerotium rolfsii* are recorded in Table XIV.

TABLE XIV.

Growth in mm. of S. rolfsii *from betel-vine at different temperatures in °C.*

Days	0°	10°	12°	17°	20°	24°	28°	32°	35°	37°	40°
1	0	0	0	0	4	8	9	7	6	3	0
2	0	0	0	5	18	27	33	16	24	8	0
3	0	0	4	10	37	44	55	38	37	15	0
4	0	0	5	15	58	64	81	58	59	21	0
5	0	0	6	26	74	83	89	80	74	30	0
6	0	0	7	35	88	89	..	89	85	42	0
7	0	0	10	47	..	..	..	..	89	53	5
8	0	0	12	58	..	..	..	..	..	58	7
9	0	0	15	68	..	(Growth here covered the				61	10
10	0	0	19	77	..	entire Petri dish)				66	10
11	0	0	23	84	..	..	..	..	..	67	10
12	0	2	27	89	..	..	..	..	..	67	10

The fungus attained its maximum diameter within five days at 28°C. and this seems to be the optimum for growth. Petri dishes that had been placed in an incubator adjusted to 43°C. showed no growth of the fungus and at the lower end temperatures below 10° are evidently inhibitive to growth.

A similar study of the Bengal strain of *Phytophthora parasitica* was conducted and the results obtained are recorded in Table XV.

TABLE XV.

Growth in mm. of Phytophthora parasitica *from betel-vine in °C.*

Days	0°	10°	12°	17°	20°	24°	28°	32°	35°	37°	41°
1 . . .	0	0	0	0	10	14	15	0	0	0	0
2 . . .	0	0	5	11	19	29	34	21	12	0	0
3 . . .	0	0	9	17	30	45	53	39	23	0	0
4 . . .	0	0	14	26	42	60	75	55	29	0	0
5 . . .	0	5	18	36	50	77	86	71	34	0	0
6 . . .	0	8	22	42	58	88	89	86	39	0	0
7 . . .	0	10	26	50	64	89	..	89	43	0	0
8 . . .	0	12	31	58	75	..	..	..	47	0	0
9 . . .	0	14	36	64	88	..	..	..	50	0	0
10 . . .	0	16	40	75	(Growth here covered the entire dish)				53	0	0
11 . . .	0	18	45	88					55	0	0
12 . . .	0	20	50	..	..	..	..	..	57	0	0

Growth in the Petri dishes placed at 28° was always ahead of that at other temperatures and this is about the optimum for the growth of the fungus. At and above 37° there was no growth and temperatures below 6° in the refrigerator inhibited the growth of the fungus also.

To determine the thermal death point of the three fungi six matched test tubes of Quaker Oats agar where the mycelium of the respective fungi half covered the area were selected. In a seventh tube there was only agar in which was thrust an accurately calibrated thermometer. These tubes were placed in a thermostat at constant temperature. When the thermometer indicated that the agar had

reached the temperature of the thermostat, they were left there for five minutes and then withdrawn. These test tubes were placed in incubators that were adjusted to temperatures for the optimum growth of the three fungi. The table below records the results obtained.

TABLE XVI.

Thermal death point in °C. of foot-rot fungi of betel-vines

Fungus	46°	47°	48°	49°	50°	51°	52°	53°	54°	55°
P. solani . . .	6/0	6/0	6/0	2/4	1/5	0/6	0/6	..	..	..
S. rolfsii . . .	..	..	..	..	6/0	6/0	6/0	5/1	3/3	0/6
P. parasitica (Bengal) .	1/5	1/5	0/6	0/6	..	..	..	..	..	..

(This first figure indicates the number of viable test-tubes and the second the number of test-tubes in which the fungi were dead.)

From the data given in Table XVI, it will be noted that five minutes' exposure at 51°C. kills the Rhizoctonia, but *Sclerotium rolfsii* can tolerate a higher temperature, that of 54°, the next higher being its thermal death point. The Phytophthora was killed at a much lower temperature, *viz.*, 48°C.

The salient facts regarding the range of growth, optimum temperatures for growth and the thermal death points are given in the form of a summary in Table XVII.

TABLE XVII.

Temperature responses of the three foot-rot fungi of betel-vines

Fungus	Range of growth (all in degrees centigrade)	Range of good growth (all in degrees centigrade)	Optimum (all in degrees centigrade)	Temperature after continuous exposure to which the fungus is killed (°C.)	Thermal death point (°C.)
P. parasitica . . .	10-35°	17-32°	28°	37°	48°
R. solani . . .	12-37°	17-35°	26°	41°	51°
S. rolfsii . . .	10-40°	17-37°	28°	43°	55°

Some meteorological data for Calcutta and Narayanganj, which are within and at opposite sides of the delta, are given. The records of other places within the infected area do not differ much and the incidence of the disease is not known with sufficient accuracy to make an attempt at correlation possible.

TABLE XVIII.

Meteorological data for Calcutta and Narayanganj for the years 1926-1927.

	1926							1927						
Calcutta	Mean max.	Mean min.	Highest max.	Lowest min.	Humidity at 8 A.M.	Days on which rain fell	Rainfall	Mean max.	Mean min.	Highest max.	Lowest min.	Humidity at 8 A.M.	Days on which rain fell	Rainfall
May . .	96·5	76.7	102·1	72·2	78	6	5·09	94·7	77·8	98·7	69·7	81	6	4·91
June . .	96·6	81·7	107	75·3	81	5	6·31	92·3	80·2	101·4	74·7	86	11	11·46
July . .	89	78·9	97·2	74·6	90	20	25·37	89·2	79·2	93·9	75·6	88	13	8·48
August .	87·6	79	91·1	75·5	91	18	21·21	88·8	79	92·7	76·6	89	15	7·02
September .	89	71·1	93·8	76·1	88	12	6·12	90·1	78·6	92·6	76·1	89	12	7·04
October .	87·9	74·6	91·2	66·2	83	7	4·01	89·8	75·3	92·3	68·5	85	4	2·92

	1926							1927						
Narayanganj														
May . .	91·9	76·8	97·5	67	78	8	6·54	90·3	74·5	94·7	57·8	84	10	7·43
June . .	92·1	79·5	95·4	73·1	85	8	5·56	91·7	78·3	95·7	72·8	84	10	4·24
July . .	87·9	78·3	90·5	76·4	87	17	10·04	89·1	78·6	92·5	76	86	16	10·16
August .	87·8	78·3	91·6	75·8	88	19	15·14	88·9	78·5	93·5	74·2	86	13	4·07
September .	88·6	78	95·4	74	89	13	17·75	88·6	78·3	92·3	75·4	87	10	4·61
October .	88·4	75	93·5	65·8	86	9	11·45	89·5	75·6	93·7	69·9	85	9	3·51

An examination of the data recorded in Table XVIII will show that the year 1926 was a wetter year with heavy rainfall and considerable flooding while the year

1927 was comparatively drier with a lack of flooding. The disease was much spoken of during the former year and was also said to be rather severe while in 1927 there was less disease. In the experimental plots in four places in the delta and the neighbouring *borojes* there were certainly far fewer deaths in 1927.

During the monsoon when the humidity is continuously high the minimum and maximum daily temperatures are within the range at which the three fungi grow well. For instance the lowest temperature recorded in the table is 65·8°F. or about 19°C. and it is within the range of good growth of all the three fungi. The highest temperature recorded in the same period is 107°F. or 41·7°C. At this temperature both *Phytophthora parasitica* and *Rhizoctonia solani* are killed provided they are exposed to this temperature continuously for twelve days. This high temperature however does not last in the course of a day for more than three hours. In the case of *S. rolfsii* this temperature does not kill the fungus though its growth is inhibited by it.

It was of interest to see whether growth of the three fungi would take place after they had been exposed to 41·7°C. for three hours. A test was therefore made in Petri dishes. One set of dishes was not exposed to this temperature while three others were exposed for one, two and three hours respectively. After exposure they were placed on the laboratory table where the temperature was between 31 and 33·5°C. The data are recorded in Table XIX.

TABLE XIX.

Growth in mm. of three foot-rot fungi that had been exposed to 41·7°C., for nil, one, two and three hours respectively.

Exposure (hours)	*P. parasitica*				*R. solani*				*S. rolfsii*			
0	21	50	76	93	27	57	92	95	14	40	74	93
1	21	45	71	89	24	57	87	94	5	40	73	91
2	21	48	74	87	24	53	87	84	9	38	68	91
3	22	44	80	90	26	56	86	92	8	18	42	53

Excepting a slight inhibition in the growth of *S. rolfsii* it will be noted that an exposure of three hours to 41·7° does not have any influence on the growth of the fungi.

During the hot weather the betel-vine plants are kept in moist air under dense shade and though the conditions of temperature are comparatively unfavourable

for growth of the fungi, they are never so unfavourable as to cause growth to cease. This is borne out by the occurrence of deaths in the field. During the cold and hot weathers which are both comparatively dry, deaths are few. During the monsoon, however, deaths occur in large numbers for temperature and moisture are both favourable to growth.

Control measures.

As *Phytophthora parasitica* (Bengal strain) is the most important of the three causal organisms of foot-rot, considerable attention has been given by the Bengal Government Department of Agriculture to reduce or control the damage caused by this fungus. In the betel-vine *borojes* that have been examined clean weeding is the rule and the amount of shade is regulated according to the season and there is little to be changed here.

Seeing that the ill effects were greatest on the stems about ground level it seemed wrong to have a mass of from ten to twenty stems parallel to and in contact with one another along the slight depression on top of the ridges. Attempts were therefore made to grow each vine on its original root system. When the apex of the vine reached the root of the *boroj*, the stem was either coiled and tied to supports or tied horizontally about a foot above ground level. In both cases the vines grew comparatively slowly, produced fewer leaves that were small in size and a large number withered gradually and died during the hot dry season, so that though the vines were less attacked by the disease, these defects rendered the method of no practical value.

Steeping the cuttings and spraying the horizontal stems and the lower portions of the upright vines three or four times with resin Bordeaux mixture, 2·5—2·5—50, just before the rains set in, in June and once a month thereafter, has reduced or controlled the disease where there is not excessive flooding. Where however flooding is frequent or continuous for a long time, no real success has occurred. Dastur [1927] has had similar experience in the Central Provinces where he has found that the application of Bordeaux mixture, 2—2—50, completely controls the disease. The conditions there, however, are simpler as the betel-vine cultivator has not to contend with the degree of flooding so prevalent in Bengal.

Leaves that have been sprayed with Bordeaux mixture are said to give a bitter taste to '*pan supari*', the preparation made from the betel-vine leaves by those who chew. Leaf spots caused by Phytophthora have been seen in the fields on five occasions and it is reasonable to assume that the leaves are so seldom attacked that omission to spray them will have little adverse effect on the efficacy of control provided diseased leaves are hand picked as soon as the spots appear. Accord-

ingly the lower portions of the upright vines to a height of one or two feet and as much as possible of those lying horizontally were sprayed. It appeared that the sprayed vines had deeper green leaves than the unsprayed ones and in the opinion of the '*barois*' they were also more vigorous.

At Banhoogli the dying and dead vines in a heavily infested *boroj* were removed in 1925, straw was burned on the soil and the land was trenched and replanted. In that year there were 763 or about 10 per cent. deaths. Next year the vines were sprayed with Burgundy mixture three times and there were 2,300 deaths or about 30 per cent. In 1927 they were sprayed with Bordea ux mixture, 5—5—50 three times from June to August and there were no deaths. This was a year of low rainfall and little flooding and the disease was less in intensity everywhere. It was noticed that the Bordeaux mixture of this strength retarded the bursting of the buds and the early growth of the cuttings and also the growth of the vines so the strength was reduced to half. Three sprayings were given each season during the next four years and there were practically no deaths. The adjoining *borojes* that were reckoned as ckecks went out of betel-vine cultivation as the vines had all died in the meanwhile. Other betel-vine gardens had ten to thirty two per cent. disease every year except in 1927. With similar treatment the disease caused by Phytophthora had been eliminated at Chinsurah and reduced at Bira and Dacca. At Santragachi in a low lying *boroj* subject to flooding several times and for considerable periods during each year, spraying with Bordeaux mixture made no difference and it is significant that in 1927 a year of short rainfall and hardly any flooding, the number of deaths here was least. When there is standing water about the horizontal stems at a time when conditions for the growth of Phytophthora are near the optima, the chances for the zoospores reaching suitable infectible places on the vines are very great, resulting in a sudden increase in the number of deaths within the *boroj* and in the amount of infective fungous material that passes from *boroj* to *boroj*. It would appear then that the disease caused by *Phytophthora parasitica* cannot be reduced in such low-lying areas by spraying and that the vines will have to be grown on higher land or on land raised beyond flood level. Such a practice is noticeable in betel-vine growing areas of the Gangetic plain in Bihar and the United Provinces of Agra and Oudh where it is the common custom to place betel-vine *borojes* on low hillocks raised a few feet above the surrounding plain.

The readiness with which all the three fungi can be carried by water at some stage of their life-history, notably in the sporangial stage of the Phytophthora and in the sclerotial stage of the Rhizoctonia and the Sclerotium, renders methods of sanitation of the greatest importance. It is necessary to remove carefully all dying and dead parts of the vines and to destroy them. It is not enough to remove a

wilted vine from the place on the stem where it breaks easily but the discoloured stem must be traced back along the horizontal portion and the diseased part wholly removed. Otherwise the fungi travel along the horizontal stem from the diseased end left in the ground and attack the next upright shoot, then the next and so on, besides invading other stems that lie in contact and in this way soon destroying a whole row of plants. It is necessary also to exercise great care during the process of removal, so that healthy plants are not touched by the diseased plants or by the hands of the worker. To throw away the diseased parts but aggravates the trouble by allowing an opportunity for continued growth and for contamination of the water supply of his own and his neighbours' *borojes*. The infected material must be destroyed and the only available effective way to do this is by burning and this can be done easily as the amount of diseased material that will be collected at a time from an infected *boroj* is small.

During the spraying trials with varieties grown in Bengal, Dholdoga, Bhubna, Kanka, and Bhangla, it was early noticed that the number of deaths of the variety Bhubna was relatively less. It is possible that this variety has some resistance to the disease but this has yet to be tested thoroughly. The *baroi* however has been sufficiently impressed with the results so far as to be replacing Dholdoga, the favourite variety, by Bhubna. If this surmise proves to be correct then there is hope of controlling the disease in the wetter portions of the delta.

In the last few years Rhizoctonia has in some places caused a very considerable number of deaths soon after the rains and in the early cold weather. Spraying with Bordeaux mixture has been ineffective on this foot-rot and a trial was therefore given to soil disinfectants. Preliminary trials with a liquid compound known as Kerol showed some promise. It was added to Quaker Oats agar to make twenty different concentrations, between 0·002 and ·714 per cent. by volume and the highest concentrations at which the three foot-rot fungi, Phytophthora, Sclerotium and Rhizoctonia, grew were 0·015, 0·018 and 0·048 per cent. respectively. As a conveniently workable method a concentration of one part in 1400 of water (0·07 per cent.) was chosen as it kills the fungus wherever it comes in contact with it without at the same time affecting the vines. Applied to the soil, the mycelium in the soil is killed but not the mycelium within the infected stems that have not decayed and disintegrated. Such infected pieces of stems must be picked by hand and removed in order that this method of prevention may become efficacious.

Sclerotium rolfsii appears so seldom in Bengal that no preventive measures have been undertaken against it as such but its reaction to a soil disinfectant is also being examined. When the investigation in Bengal is finished it is likely that preventive measures against all these fungi will consist most of all in growing the vines on land

above the flood level, and in the exercise of care in removing and destroying dying and dead parts of the plant as soon as they appear and secondly in treating the ridges where the horizontal stems lie with a soil disinfectant and in spraying these stems and the bottom parts of the upright stems with Bordeaux mixture.

The cultivation is already most intensive and the profits are such as to render the extra cost of material and labour in protecting the crop well within the compass of the grower.

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SUMMARY.

Foot-rot diseases of betel-vines have been prevalent in Bengal for a considerable number of years but recetly losses due to these have assumed considerable proportions. The vines are usully grown in low-lying areas of the delta subject to almost continuous flooding in the monsoon at which time most of the damage is done.

The lesions are first observed at the foot of the vines at ground level, and the darkening of the stem which follows may extend to six inches above ground level. The stem at this place rots causing a wilt in the rest of the plant. The disease is however localised to the 'foot' at the darkened portion only.

Several fungi are associated with the diseased plants of which a Rhizoctonia, a Sclerotium and a Phytophthora have been proved to be pathogenic. *Glomerella cingulata* (Stnm.) Spaul. et Schr., has not of itself been induced to produce disease though when it is placed on a lesion caused by the Phytophthora, it grows rapidly and causes some damage.

The Rhizoctonia and Sclerotium have been identified as *R. solani* and *S. rolfsii* and of these the latter is relatively unimportant in Bengal. *R. solani* causes damage to the vines soon after the rains and in the early part of winter.

Much of the loss however is due to Phytophthora in Bengal. This and the Malaya strain of the fungus that causes a similar disease to vines there, have been found to be referrable to the species *Phytophthora parasitica* but a Phytophthora from the Madras Presidency where it causes also a foot-rot in betel-vines seems to be different on morphological (sporangial measurement) grounds. Its systematic position remains to be determined. For analysing the spore measurement data of these fungi, the co-efficient of racial divergence was used by Professor Mahalanobis to whom they were sent for analysis.

Temperatures between 20° to 30°C. seems to be most favourable for the growth of these fungi, *R. solani* being favoured by temperatures nearer the cooler end while *S. rolfsii* and *P. parasitica* being favoured by slightly higher temperatures. The preference of *R. solani* for slightly cooler temperatures is in consonance with the field observations, for the betel-vines suffer from disease caused by this fungus only after the rains and in early winter.

The thermal death point for *R. solani* is about 51°C. for *S. rolfsii* 55° and *P. parasitica* 48°.

Spraying the lower parts of the stems of betel-vines with Bordeaux mixture (2·5—2·5—50) beginning before the monsoon and three times at intervals of a month afterwards controls the disease due to *Phytophthora parasitica*.

For the disease due to *R. solani* the application of a weak solution of a fungicide, Kerol, has brought the disease under control. Growing the vines on land above flood level, removing diseased plants and applying these two fungicides control the foot-rot diseases in Bengal.

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THE CLASSIFICATION OF THE RICES OF BIHAR AND ORISSA.

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I. INTRODUCTION.

With the possible exception of China for which no statistics are available, India is the largest rice-growing country in the world; of the Indian Provinces, Bihar and Orissa has the second largest acreage under rice, being exceeded only by Bengal (Table I.)

TABLE I.

Area under rice in the several provinces of British India in 1930-31.

No.	Provinces	Area in acres
1	Bengal	20,582,000
2	Bihar and Orissa	13,927,000
3	Burma	13,047,566
4	Madras	11,677,529
5	United Provinces	6,843,572
6	Central Provinces and Berar	5,541,208
7	Assam	4,651,748
8	Bombay	3,260,416
9	Punjab	976,529
10	Coorg	82,822
11	North-West Frontier Province	40,668
12	Ajmer-Merwara	416
13	Manpur-Pargana (C. I.)	144
14	Delhi	50
	Total	80,631,668

Summer, autumn and winter rices are cultivated and the amounts of each cropped in the various districts of the province are shown in Table II.

TABLE II.

Area under rice in the several districts of Bihar and Orissa, 1932-33.

No.	District	Winter rice	Autumn rice	Summer rice	Total
1	Cuttack	906,100	149,900	13,000	1,069,000
2	Ranchi	284,700	692,600	800	978,100
3	Bhagalpur	793,900	136,100	300	930,300
4	Santal Parganas	662,600	236,100	4,300	903,000
5	Balasore	878,000	14,000	1,900	893,900
6	Muzaffarpur	830,700	39,500	100	870,300
7	Gaya	833,700	8,800	..	842,500
8	Champaran	524,500	250,000	800	775,300
9	Sambalpur	258,300	517,000	..	775,300
10	Puri	601,900	17,500	9,200	628,600
11	Purnea	496,500	125,700	1,600	623,800
12	Shahabad	562,700	42,800	300	605,800
13	Darbhanga	463,400	34,800	1,200	499,400
14	Hazaribagh	146,000	345,000	..	491,000
15	Singhbhum	157,600	315,000	..	472,600
16	Manbhum	244,400	130,800	..	375,200
17	Patna	352,200	6,300	200	358,700
18	Monghyr	300,700	37,000	1,000	338,700
19	Saran	235,400	80,200	5,000	320,600
20	Palamau	43,400	132,500	..	175,900
21	Angul	125,900	18,500	..	144,400
	Total Bihar and Orissa	9,702,600	3,330,100	39,700	13,072,400

From this table it is evident that winter rice is easily the most important, and autumn rice is grown mainly in those districts, *e. g.*, Ranchi, Sambalpur, Hazaribagh and Singhbhum, where the conditions do not permit of a long duration crop. Summer rice is grown in a few scattered localities.

The present work was commenced in 1928 at the suggestion of Mr. G. S. Henderson, the then Director of Agriculture, Bihar and Orissa. In order to obtain as large a number of varieties as possible in a short space of time members of the field staff of the Botanical Section at Pusa were sent on tour all over the province to collect single plants of all the varieties grown by cultivators. Nearly 1200 heads from single plants were obtained in this way but after discarding duplicate, etc., only 123 types finally remained for study and these together with their districts of collection are enumerated in Table III.

TABLE III.

Types isolated from the several districts of Bihar and Orissa.

Bhagalpur	7, 12, 15, 23, 32, 39, 59, 64, 74, 75, 84, 88, 92, 93, 102, 104.
Champaran	3, 16, 18, 19, 21, 34, 41, 47, 49, 55, 57, 69, 87, 90, 100, 105, 119.
Cuttack	58, 99.
Darbhanga	2, 4, 11, 14, 17, 20, 25, 26, 29, 31, 35, 37, 40, 42, 43, 44, 45, 46, 48, 50, 51, 52, 53, 54, 60, 62, 63, 67, 68, 70, 77, 78, 80, 81, 89, 94, 95, 96, 97, 98, 106, 108, 109, 111, 113, 114, 115, 116, 117, 118, 121, 122.
Gaya	24.
Hazaribagh	10, 13, 38, 71, 78, 82.
Manbhum	27, 28, 30, 86.
Muzaffarpur	1, 5, 6, 8, 9, 22, 65, 79, 101, 107, 110, 112, 120, 123.
Patna	36, 61, 83, 85.
Purnea	66, 91, 103.
Ranchi	33, 56, 72, 76.

In the absence of any previous taxonomic work on the rices of Bihar and Orissa it was obvious that the classification of types would prove both useful and interesting.

As often happens in making such classification, one great difficulty encountered was the fact that the same variety may bear different names in different localities.

For example the two " varieties " in each of the following pairs were found to be identical :—

Parikarma of Purnea and Thakur Prasad of Muzaffarpur.

Macchadali of Darbhanga and Tulsimukand of Hazaribagh.

Kamode of Darbhanga and Ratansar of the same district.

Occasionally the names are indicative of the characteristics of a variety. Thus :—

Kalam dhan=grain long and pointed like a pen.

Tulsi Phul=purple like the medicinal plant *tulsi* (*Ocimum sanctum*).

Parva Pankh=winged like a pigeon.

Basmati=scented.

Amma ghaur=bunched like a bunch of mangoes.

Copeland [1924] states that 8000 varietal names have been recorded in India but there is no reliable estimate of the number of varieties that are distinct. The latter are no doubt far less than the total array of names but the number of varieties is probably still very large and this plethora is in all likelihood to be attributed to the fact that the rice plant is subject to extreme ecological specialization and that different varieties are required for the very varied conditions obtaining even in parts of the same province.

II. Brief review of previous classifications.

The first serious attempt at a classification of the varieties of rice was probably that of Kikkawa [1912] who based his classification primarily on agricultural characters and secondarily on characters of grain size. Graham [1913] used colour of leaf sheath and kernel and then made further subdivisions on the basis of grain size and other characters such as length of life-period, extent of exsertion of peduncle, disposition of rachis and branches, shape of glume, type of facets, etc. Beale [1927] classified the rices of Burma primarily on the constitution of the rice kernel, *i.e.*, whether glutinous or starchy, and secondarily on grain size, colour of stigma, apiculus and leaf sheath. Thadani [1927] based his scheme firstly on the character of awning, subsequently subdividing the main groups thus formed on the basis of colour characters and finally on grain size. Sethi [1930] followed Kikkawa in grouping various types according to grain size, using kernel characters for further subdivision. Lastly, Mitra and Ganguli [1931] classified rices according to the glutinous or starchy character of the kernel, the presence or absence of awns, grain size, colour of kernel and colour of inner glume, using the characters in the order named.

III. Bases of classification and their comparative merits.

The need for a really adequate classification of the varieties of rice was voiced at a Rice Congress at Valencia [Copeland, 1924] in the form of a resolution to the

effect " that there be made in all countries a botanical study of the varieties of cultivated rice, seeking a provisional classification, based on the characters which may be considered fixed. That when one or other of these characters shall be recognised, the stations notify those of other countries busy with studies of this kind, for the purpose of attaining uniformity of method."

The first desideratum of a satisfactory classification is that it should be natural, *i.e.*, it should indicate as far as possible the true genetic relationships of the forms classified. Unfortunately in the present state of knowledge very little is known about the kinship of the vast number of rice varieties in cultivation, but the various plant characters and their claims to a place in a system of classification may be discussed.

For convenience plant characters may be divided into morphological and physiological characters. The latter are of great importance but they have not yet been sufficiently studied, and it is difficult to assess and express them in terms sufficiently definite to be of value for taxonomic purposes. It is therefore the morphological characters that are usually employed: they may be divided into qualitative and quantitative characters. Though the mode of inheritance of a large number of quantitative characters has been elucidated, it is often complex, depending on the action and interaction of several factors. Because of this and in consideration of the fact that their expression is often easily modified by environment, they are as a rule, not so satisfactory to use as the qualitative characters. Characters used for classification should as far as possible be " fixed," *i.e.*, not subject to wide variations with changes of soil and season, clear-cut and easily observed, and should lend themselves to ease of description. The qualitative characters, and particularly the colour characters, generally fulfil these requirements. Colour, mainly due to the presence of anthocyanin, may manifest itself in almost any part of the rice plant and the presence or absence of colour affords one of the most convenient criteria for the discrimination of types.

The colour characters of the rice plant have been studied by certain workers notably by Parnell [1917, 1922] and Hector [1916, 1922] and their work has revealed that there is often a strong linkage between some of these characters. Parnell found associations of:—

1. Gold internode and gold inner glume.
2. Purple-lined internode and purple inner glume.
3. Purple node and purple pulvinus.
4. Purple-lined internode and purple sheath.
5. Purple stigma and purple axil.
6. Purple internode and inner glume, and white stigma and axil.

7. Gold internode and glume, and purple stigma and axil.
8. Purple stigma, axil, and apiculus.*
9. Pigment in the plant and red rice.
10. Lack of pigment in the plant and grey brown rice.

However we have noted numerous exceptions to some of the above and these are detailed below:

Characters found to be associated by Parnell	No. of types in which these characters are associated	No. of types where the association does not exist
1. Gold internode and gold inner glume	18	1
2. Purple-lined internode and purple inner glume	5	3
3. Purple node and purple pulvinus	11	8
4. Purple-lined internode and purple sheath	21	6
5. Purple stigma and purple axil	22	5
6. Purple internode and inner glume, and white stigma and axil.	1	4

Hector [1922] found associations between the colour of inner and outer glumes and that of the ligule and between white rices and colourless ligules though the reverse of these does not hold true. In the present collection 9 exceptions to the first association have been found. It has been observed that colour of leaf is associated with that of auricles, pulvinus and leaf sheath. Graham [1913] stated that colour in leaf sheath is usually associated with a coloured apiculus. This has been found to hold true except in the case of 9 types.

In describing the associations that may be found, Hector stated that certain colour combinations do not occur; these are given below:

Combination	1	2	3	4	5	6	7	8	9	10	11
Leaf-sheath					X			X			
Apiculus						X					
Internode	X				X			X	X		
Stigma	X				X			X			
Inner glume		X		X						X	
Outer glume		X		X		X					
Ligule			X								X
Pulvinus							X		X		
Auricle							X		X		

(X=Coloured).

* Restricted apiculus. (Section III, para. 16.)

Three exceptions were found to No. 6; that is, three types were found with colour in the apiculus and outer glume. Some of the characters of the paddy plant may now be considered in detail and their relative merits as possible bases of classification discussed.

Qualitative characters.

1. Starchy and glutinous kernels.—Rices may be divided into two main groups, those with starchy grains and those with glutinous grains, the former being designated by Kikkawa [1912] as *O. sativa utilissima* and the latter as *O. sativa glutinosa*. The glutinous endosperm is said to contain only a small percentage of common starch and a considerable proportion of soluble starch and dextrine besides some maltose. On account of this, glutinous rices form a pasty mass if cooked in the ordinary way.

2. Long and short outer glumes.—Most rices have a short outer glume but in a few types the outer glume is equal in length to the inner glume and palea. These classes are easily separated and afford a convenient basis of discrimination.

3. Single and double grained spikelets.—The occurrence of multiple ovaries giving double grained spikelets was reported by Prain and subsequently by Watt [1891]. This is a useful character for classification.

4. Solitary and clustered spikelets.—In a few types the spikelets are clustered. By hybridization forms intermediate between clustered and non-clustered have been obtained [Ramiah, 1931], but the distinction is clear enough in "natural" varieties.

5. Long and short internodes.—Some deep water types, for example T. 9, are characterised by long internodes with production of roots at the aerial nodes; the depth of water decides to a great extent the production of roots from the several nodes. The growth of these paddies keeps pace with the water when it rises but on its fall the crop lodges as the sub-aerial root system is inadequate to support the abnormal height of the plant.

6. Leaf-sheath colour.—The leaf sheath is said to be coloured when the pigment is present in the outer vascular bundles, and parenchyma of the leaf sheath, while colour in the inner bundles is separately distinguished as coloured "axil" and is dealt with below. The colour varies from green to shades of purple. In Types 57-59, 61-64, 67, 92, 101, 116-118, 120 and 122, the purple colour is restricted to the lower half of the sheath, whereas in Types 9, 65, 66, 79, 93-96, 102 and 123 the colour spreads over the entire length.

Hector [1916] did not distinguish between leaf sheath and axil in respect of colour, but, as Parnell [1917] has indicated, the presence of colour in these two parts

is governed by separate factors. In the present study it was found that they are not always similar to one another but may behave differently in inheritance. As Graham [1913] pointed out colour in the leaf sheath is usually associated with colour in the apiculus. Of these two the latter character has been preferred as it can be more conveniently determined.

7. Axil colour.—The axil is said to be coloured when purple pigment is present in the inner vascular bundles and the parenchyma of the inside of the leaf sheath. Types with green leaf sheaths usually have white axils but types 56 though possessing a green leaf sheath has a purple axil. Similarly, types with purple leaf sheath have purple axils except in Types 67, 79, 103 and 122, where the axil is white. As colour in the axil is linked with colour in the stigma [Parnell, 1917] and the latter is associated with colour in the stem [Hector, 1922] which is used in the classification, it does not appear to be necessary to make use of the axil character.

8. Colour of pulvinus and auricles.—The pulvinus is usually greenish white and the colour when present varies from minute dots as in Types 9, 58, 59, 65, 66, 92-96, 102, 120 and 123, to a uniform spread of purple in Type 67. The auricle is studded with purple dots in Types 9, 58, 65-67, 92-96, 102 and 120.

9. Ligule colour.—The ligule is usually non-pigmented but in Types 92, 93 and 96 it is studded with purple dots, and is uniformly purple in Types 9, 66, 79, 94 and 95.

The characters of the pulvinus, auricle and ligule are difficult to use in classification because of the numerous gradations in pigment varying from minute dots to a uniform spread of intense purple.

10. Leaf colour.—Leaves are in most cases green though different shades of green can be discriminated. When purple is present it is more pronounced at the base and along the margins in Types 9, 94, 115, 116, 118 and 121, while in Type 79, the leaves are uniformly deep purple.

11. Internode colour.—Green internode is associated with green leaf sheath and purple internode with purple leaf sheath. As, except in the very rare cases where the internode is completely enclosed by the sheath, there is very little difficulty in estimating its colour, it is more easily distinguished than colour in pulvinus, auricles, ligule, node and axil. For the sake of simplicity only the broad distinction into groups with green and purple internodes has been adopted though it is possible to distinguish between the grades of purple and between the presence of colour in lines (*i.e.*, in vascular bundles only) and its uniform spread.

12. Node colour.—Colour in the node varies by imperceptible degrees from minute dots of purple to a uniform deep purple and as such cannot be usefully

employed for classification. Purple node is associated with purple leaf sheath, but when the node is green the sheath may be either purple or green. Types 8, 9, 56-58, 61, 63-66, 92-95, 102, 120, 123 have purple nodes.

13. Septum colour.—The colour on the lower surface of the diaphragm inside the stem at the node varies from shades of cream through orange to purple. Varieties with a colourless apiculus usually possess a cream septum, while brown colour in the apiculus is associated with a dark cream colour in the septum. When the stem is gold, the septum is either orange, or orange at the lower nodes and purple at the higher ones, according as the apiculus is coloured or colourless. Since septum colour is largely associated with colour in the apiculus which is given a prominent place in the classification, it has not been used.

14. Outer glume colour.—In most types the outer glumes are small, white, stiff and shiny. They are long in Types 3, 4, and 5 and reference has already been made to them in Para. 2 (P. 624). Types 46-54 and 106-112 have brown outer glumes while Types 65, 68, 79, 103, 113-121 have purple outer glumes; both these colours fade to lighter shades at maturity.

Coloured outer glumes are associated with the coloured "spreading apiculus" discussed in detail under the character of apiculus.

There is no difficulty in distinguishing or describing colour in the outer glumes as three distinct colour groups exist, *viz.*, white, brown and purple. The absence of merging shades of colour recommends the use of this character in classification.

15. Inner glume colour.—The inner glume and palea which will be referred to as inner glume throughout this paper, shows the greatest variety of colour of any organ in the rice plant, ranging from straw through shades of gold, brown and purple to black; these colours are quite definite and can be distinguished with ease. The nomenclature adopted in describing them follows as far as possible that of previous investigators, and the following colours are recognised:—

(*a*) *Straw.*—The word "straw" has been selected to indicate the colour of the inner glume in most paddy varieties where it is of the same colour as that of the dry straw. Since this colour is neither absolutely white nor yellow (as Hector [1922] and Mitra [1927] have described it) but a blend of both these colours, it is conveniently termed "straw". Under this name are also included the "ripening straw" and "ripening gold" of Parnell [1922] as in both these cases the occurrence of transient dirty specks and yellow colour, before the maturation of the grain whereby they are respectively distinguished from straw, is perceptible only by a very detailed field study throughout the flowering period and hence these two colours cannot readily be adopted for classification.

(*b*) *Gold.*—This group contains types ranging from light shades of coppery yellow to dull dark gold. Within this group may be distinguished :

"Tip and base" gold. This denotes the absence of gold colour in the extreme tip and base of the glume as opposed to the uniform spread of colour typical of the group.

"Piebald" gold. This is like "tip and base" gold but the gold colour is restricted to the middle one-third of the glume, leaving a broader band of straw colour at the top and bottom.

(*c*) *Olive.*—This term is used to indicate the development of brownish specks soon after flowering, which intensify to different shades of gold brown. In some types the colour is distributed uniformly over the ridges and furrows and this is called "uniform" while the appearance of colour in furrows only is described as "furrowed". "Piebald" as in the case of the gold group denotes the localization of olive colour to the middle of the glume, leaving the top and bottom free.

(*d*) *Brown.*—Here are placed those types in which a brown pigment grows over an initial lemon yellow colour resulting in a reddish brown tint when the grain attains maturity ; it fades eventually to a dull brown colour.

(*e*) *Purple.*—This group is characterized by the presence of purple pigment and may be further divided into three sub-groups according as the colour develops before or after flowering or at maturity.

(*f*) *Ripening black.*—As the name suggests, this refers to the development of black colour as the grain attains maturity. The colour begins as a smoky coat over the glume, gradually deepening into black and finally fades.

The maximum expression of the colour of the inner glume being taken as the primary basis of classification, the rices may be divided into two groups—those with colour characters recessive to straw, and those with colour characters dominant to straw.

Colours recessive to straw : { Gold. / Olive.

Colours dominant to straw : { Brown. / Purple. / Black.

The colour characters dominant to straw have been found to be brought about by one, two or more factors [Parnell, 1917, 1922 ; Illiffe, 1927-28 ; Mitra, 1927 ; Nagai,

1921]. The colours recessive to straw were found to be due to the interaction of certain inhibitory factors [Parnell, 1917, 1922, Hector, 1922].

It is possible that the colour of the inner glume may be sometimes correlated with certain physiological characters. This is suggested by the fact that all the gold glume types examined have a greater percentage of unset spikelets than other glume colour types.

As it affords an extremely convenient unit for classification the inner glume colour has been used as the principal criterion of classification, after the preliminary division into 5 main classes.

16. Apiculus colour.—The presence or absence of colour at the tip of the inner glume is used as one of the main diagnostic features of the classification. Hector [1922] found that the colour of the apiculus was closely linked with that of leaf sheath and also with colour in internode and stigma.

It was found that in some types the colour is restricted to the extreme tip of the glume while in others it spreads a little way down the glume. The latter is here termed as "spreading apiculus" and has not been distinguished by previous workers; it was found to be associated, in this collection, with colour in the outer glume, in both the brown and purple groups. In the case of the "restricted apiculus" the purple colour is correlated with purple colour in the stigma and axil, whereas with the "spreading apiculus" the stigma may be either white or purple. Next to the inner glume colour this character has been found to be most useful for classification.

17. Stigma colour.—The stigma may be either white or purple. There is a positive correlation between stigma colour and axil and leaf-sheath colour; only in Type 56 was a green leaf sheath associated with a purple stigma while in Types 67 and 116 purple leaf sheath was associated with white stigma. As the stigma colour is usually associated with colour in the apiculus which is employed in the classification, it is not used here.

18. Awns.—Perhaps no other character is so variable as the length of the awn. In different types this may vary from the smallest possible "tip" to about 3 inches or more in the same plant. Awns may be present in the bearded types in all spikelets or only in a few and are generally of the same colour as the apiculus, the pigment fading at maturity. As the character is so inconstant the gradations of awning have not been given a place in this classification.

19. Kernel colour.—With the exception of Types 1 and 2 all the varieties are starchy. In some of the bold types an opaque patch is usually found in the middle while the majority of the slender-grained rices are entirely translucent. Four colours—red, light red, dull white and white, have been distinguished in the collection

though Parnell [1917, 1922] reported many others. The genetic constitution of the first and the last types has been worked out by Parnell and is as follows:—

Red=**R A N** (in pigmented plants).

R A n (in non-pigmented plants).

White=**r a N** or **r a n** (in non-pigmented plants).

r A N (in pigmented plants).

Red rice indicates not only the presence of the factor **R** for redness; but also the presence of **A**, the colour base of the rice plant which in the presence of **N** produces the anthocyanin pigment in the vegetative parts. Rice colour appears to be also an indicator of some of the physiological attributes of a type for red rices are commonly cultivated in dry upland and alkaline soils. White rices, on the other hand, predominate among the swamp paddies.

20. Exsertion of panicle.—The length of the peduncle varies. In Types 1 and 2 the panicle is completely enclosed within the boot-leaf, while in many other types, *e.g.*, Types 15, 17, 30-32 and 35 it projects a little above the top of the sheath of the boot-leaf when it is known as " exserted ". If it projects much further as in Types 3, 10, 16 and 33 it is termed " far-exserted ". The expression of this character is liable to be greatly influenced by variations in soil and season, and it has not therefore been deemed worthy of a place in the classification.

21. Density and type of panicle.—The panicle varies considerably in length, density, and arrangement. The branches of the panicle are either simple or again divided. In the classification three broad groups are distinguished on the basis of the compact, semi-compact or spreading nature of the panicle. The existence of forms with clustered spikelets has already been alluded to.

Quantitative characters.

1. Tillering.—Tillering sometimes commences in the nursery but more generally it begins about 7-10 days after transplanting when the plants are established and continues until the appearance of the panicle, the highest number of tillers being attained sometime between the 7th and 8th weeks. The habit of the plant whether erect, semi-erect or spreading depends upon the number of the tillers and the angle in relation to the main axis at which they arise. But since tillering is markedly subject to fluctuations according to the conditions of soil, water-supply and spacing, it is not here employed.

2. Height of plant.—Though the types can be grouped into short, medium and tall classes, the height varies with the environment and so is not a very suitable criterion for classification.

3. Full bloom date.—Types 1, 2, 18, 60 and 97 require a definite period of time to flower irrespective of the date of sowing and these are known as *Bhadoi* or autumn paddies (=" periodically fixed types " of Mitra [1932]) ; they usually flower in August and September. All the other types require a particular temperature and are generally known as *Aghani* or winter paddies (=" timely fixed types " of Mittra [1932]). As this character is of considerable agricultural importance when selecting a variety for a particular area it has been incorporated into the scheme of classification. The full bloom period of the *Aghani* varieties extends from the middle of October to the middle of November and for convenience three groups have been recognised : Early (3rd week of October and earlier), Medium (4th week of October) and Late (1st week of November and later).

4. Grain size.—This character based on the length, breadth and thickness of the grain has long been recognised as one of the most important taxonomic characters, and has been accorded a prominent place in the classifications of Kikkawa [1912], Graham [1913], Beale [1917], Sethi [1930] and Mitra [1932]. As regards the effect of soil conditions on grain size, it is a common belief among cultivators and mill-owners that slender-grained varieties get gradually bolder after some years of cultivation in heavy delta soils and *vice versa*. Ramiah [1933] is of opinion that there is a certain amount of truth in this belief and considers that detailed investigations are necessary properly to assess the role of soil conditions in modifying grain size. It is however, commonly agreed that grain size probably is, among the quantitative characters, the least affected by changes in soils, seasons and manures and " it is this relative constancy of the character under varied conditions that makes the classification of rices on the basis of grain size possible".

Some workers have used the size of the hulled grain, while others have taken the dimensions of the unhulled grain for purposes of classification. But it is preferable to use the former criterion as measurements of the unhulled grain are more subject to error because of variations in the thickness of the husk.

Kikkawa, Graham and Sethi have used the $\frac{\text{Length}}{\text{Breadth}}$ index and distinguished short, long and slender classes (Kikkawa and Sethi) or course, fine and long classes (Graham). Further subdivisions within these classes were effected on the basis of length, the ultimate classes varying in number from five (Graham) to nine (Kikkawa and Sethi). Beale distinguished 5 classes using length and $\frac{L^*}{B}$ simultaneously. Thadani and Mitra have used length and breadth measurements for classification.

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*$\frac{\text{Length}}{\text{Breadth}}$

There is a little doubt that length of grain and the $\frac{L}{B}$ index provide useful criteria. It is preferable to use $\frac{L}{B}$ instead of breadth as an obvious defect in the use of the latter is that when grain is classified on this basis all sorts of shapes may be found in any particular class, suggesting that this is not a natural association. But if the $\frac{L}{B}$ index is employed in place of breadth there is revealed a gradual drift from the slender to the bold types. The relatively greater constancy of $\frac{L}{B}$ over breadth under various soil conditions [Ramiah 1931, 1933] further indicates that $\frac{L}{B}$ is the more reliable character. Thickness is also important as it helps to define grain shape but it is more convenient to divide grains into the three classes, slender, medium and bold, on eye judgement rather than to attempt to define them mathematically.

The classes adopted are as follows :—

According to length.

I. 5·0 mm. and below.
II. From 5·1 to 7·0 mm.
III. 7·1 mm. and above.

According to $\frac{L}{B}$ *index.*

a. 2·0 and below
b. 2·1 to 3·0
c. 3·1 and above.

That a correlation subsists between length of grain and shape index appears to be indicated by the figures given below where the numbers of types falling into each class are shown :—

	Length classes			
Shape index classes	I	II	III	Total
a . . .	17	13	..	30
b . . .	4	95	4	103
c . . .	1	32	26	59
Total . . .	22	140	30	192

Yield.—Though varieties with a short life period generally yield less than those with a long life period, this most complex character is so liable to fluctuations that it has not been deemed desirable to employ it in classification.

IV. Key to the classification.

Glutinous rice.
Single grained.
Short outer glume.
Solitary spikelets.
Short internode.
Inner glume—ripening black.
Apiculus—colourless.
Outer glume—white.
Kernel—red.
Internode—green.
Grain size. II. *b*. Bold . . . Type 1.

Apiculus—purple.
Outer glume—white.
Kernel—red.
Internode—purple.
Grain size II. *b*. Medium . . . Type 2.

Starchy rice.
Single grained.
Long outer glume.
Solitary spikelets.
Short internode.
Inner glume—olive furrowed.
Apiculus—colourless.
Outer glume—white.
Kernel—red.
Internode—green.
Grain size II. *b*. Medium . . . Type 3.

Inner glume—dark gold.
Apiculus—colourless.
Outer glume—white.
Kernel—dull white.
Internode—gold.
Grain size II. *b*. Bold . . . Type 4.

Inner glume—ripening black.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—gold.
Grain size II. *b*. Bold . . . . Type 5.

Starchy rice.
Single grained.
Short outer glume.
Clustered spikelets.
Short internode.
Inner glume—straw.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—green.
Grain size II. *b.* Bold . . . . Type 6.

Kernel—dull white.
Internode—green.
Grain size II. *b.* Bold . . . . Type 7.

Apiculus—purple.
Outer glume—white.
Kernel—dull white.
Internode—purple.
Grain size II. *b.* Bold . . . . Type 8.

Solitary spikelets.
Long internode.
Inner glume—straw.
Apiculus—purple.
Outer glume—white.
Kernel—white.
Internode—purple.
Grain size II. *a.* Bold . . . . Type 9.

Short internode.
Inner glume—straw.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—green.

Grain size	I. *a.*	Slender . . .	Type 10.	
		Medium . . .	Type 11.	
	I. *b.*	Slender . . .	Type 12.	
	II. *b.*	Medium . . .	Type 13	Beardless.
		,, . . .	Type 14	Bearded.
		,, . . .	Type 15	Bearded. Shorter in height than Type 14.
		Bold	Type 16	Earlier than Type 17.
		,, ...	Type 17.	

Starchy rice.
Single grained.
Short outer glume.
Solitary spikelets.
Short internode.
Inner glume—straw.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—green.

Grain size II. *c.*	Slender	Type 18	*Bhadoi.*	
	,,	Type 19.		
	,,	Type 20	Ripening straw.	
	,,	Type 21	,, ,, Grain shorter than Type 20.	
	Medium	Type 22.		
	,,	Type 23	Ripening straw.	
III. *c.*	Slender	Type 24	,, ,,	
	Medium	Type 25	Earlier than Type 26.	
	,,	Type 26.		
	Bold	Type 27.		

Kernel—dull white.
Internode—green.

Grain size I. *a.*	Bold	Type 28.	
II. *a.*	Bold	Type 29.	
II. *b.*	Bold	Type 30	Bearded.
	,,	Type 31.	
c.	Slender	Type 32.	
III. *c.*	Medium	Type 33.	

Kernel—light red.
Internode—green.

Grain size II. *b.*	Medium	Type 34.	
	Bold	Type 35.	
III. *c.*	Medium	Type 36.	
	Bold	Type 37.	

Kernel—red.
Internode—green.

Grain size II. *b.*	Slender	Type 38.	
	Medium	Type 39.	
	Bold	Type 40.	
II. *c.*	Slender	Type 41.	
III. *c.*	Medium	Type 42.	

Apiculus—brown.
Outer glume—white.
Kernel—white.
Internode—green.

Grain size I. *a.*	Slender	Type 43.	
II. *c.*	Medium	Type 44.	
III. *c.*	Medium	Type 45.	

Starchy rice.
Single grained.
Short outer glume.
Solitary spikelets.
Short internode.
Inner glume—straw.
Apiculus—spreading brown.
Outer glume—brown.
Kernel—white.
Internode—green.

Grain size I. *a.*	Slender.	Type 46.		
	Medium	Type 47.		
II. *a.*	Bold	Type 48.		
b.	Slender	Type 49.		
	,,	Type 50	Ripening straw.	
	Medium	Type 51.		
	Bold	Type 52.		
c.	Medium	Type 53.		
III. *c.*	Medium	Type 54.		

Apiculus—purple.
Outer glume—white.
Kernel—white.
Internode—green.

Grain size II. *b.*	Medium	Type 55	White stigma.
	,,	Type 56	Purple stigma.

Internode—purple.

Grain size II. *b.*	Medium	Type 57.	
III. *b.*	Bold	Type 58.	
c.	Medium	Type 59.	
c.	Bold	Type 60	*Bhadoi.*

Kernel—dull white.
Internode—purple.

Grain size II. *b.*	Medium	Type 61.	
III. *c.*	Medium	Type 62.	

Kernel—light red.
Internode—Purple.

Grain size II. *b.*	Bold	Type 63.	
III. *b.*	Bold	Type 64.	

Kernel—red.
Internode—purple.

Grain size II. *b.*	Bold	Type 65.	
c.	Slender	Type 66.	

Apiculus—spreading purple.
Outer glume—purple.
Kernel—white.
Internode—green.

Grain size II. *a.*	Bold	Type 67.	
b.	Medium	Type 68.	

Starchy rice.
Single grained.
Short outer glume.
Solitary spikelets.
Short internode.
Inner glume—olive—light olive uniform.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—green.

Grain size	I. *a.*	Medium	Type 69.	
	II. *b.*	Medium	Type 70.	Bearded.
		,,	Type 71	Beardless.
		,,	Type 72	Beardless, later than Type 71.

Kernel—light red.
Internode—green.
Grain size II. *b.* Medium . . . Type 73.

Kernel—red.
Internode—green.
Grain size II. *b.* Medium . . . Type 74.

Kernel—white.
Internode—green.
Grain size III. *b.* Bold . . . . Type 75.

Kernel—dull white.
Internode—green.

Grain size	I. *a.*	Medium . . .	Type 76.
		Bold	Type 77.
	II. *b.*	Bold	Type 78.

Apiculus—purple.
Outer glume—purple.
Kernel—red.
Internode—green.
Grain size II. *a.* Medium . . . Type 79.

Inner glume—olive—olive uniform.
Apiculus—colourless.
Outer glume—white.
Kernel—dull white.
Internode—green.
Grain size II. *b.* Bold . . . . Type 80.

Inner glume—olive—olive furrowed.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—green.
Grain size II. *b.* Medium . . . Type 81.

Starchy rice.
Single grained.
Short outer glume.
Solitary spikelets.
Short internode.
Inner glume—olive—olive furrowed.
Apiculus—colourless.
Outer glume—white.
Kernel—dull white.
Internode—gold.
Grain size II. *b*. Medium . . . Type 82.
Kernel—light red.
Internode—green.
Grain size II. *b*. Medium . . . Type 83.
III. *c*. Medium . . . Type 84.
Inner glume—olive—dark olive furrowed.
Apiculus—colourless.
Outer glume—white.
Kernel—dull white.
Internode—green.
Grain size II. *b*. Bold . . . . Type 85.
Internode—gold.
Grain size II. *a*. Bold . . . . Type 86.
Inner glume—olive—piebald olive.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—gold.
Grain size II. *b*. Bold . . . . Type 87.
Inner glume—gold—light gold.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—gold.
Grain size II. *b*. Slender . . . Type 88.
Medium . . . Type 89.
Kernel—dull white.
Internode—gold.
Grain size II. *a*. Bold . . . . Type 90.
Kernel—red.
Internode—gold.
Grain size III. *c*. Bold . . . . Type 91.
Apiculus—purple.
Outer glume—white.
Kernel—white.
Internode—gold.
Grain size II. *b*. Medium . . . Type 92.
Internode—purple on gold.
Grain size III. *b*. Medium . . . Type 93.
Bold . . . . Type 94.

Starchy rice.
Single grained.
Short outer glume.
Solitary spikelets.
Short internode.
Inner glume—gold—light gold.
Apiculus—purple.
Outer glume—white.
Kernel—dull white.
Internode—purple on gold.
Grain size II. *b.* Bold . . . . Type 95.
Kernel—light red.
Internode—purple on gold.
Grain size III. *c.* Bold . . . . Type 96.
Apiculus—spreading purple.
Outer glume—purple.
Kernel—white.
Internode—gold.
Grain size III. *c.* Slender . . . Type 97. *Bhadoi.*
Inner glume—gold.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—gold.
Grain size II. *b.* Medium . . . Type 98.
Kernel—light red.
Internode—gold.
Grain size II. *b.* Medium . . . Type 99.
Inner glume—dark gold.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—gold.
Grain size II. *a.* Bold . . . . Type 100.
b. Medium . . . Type 101.
Apiculus—purple.
Outer glume—white.
Kernel—dull white.
Internode—gold.
Grain size II. *c.* Medium . . . Type 102.
Apiculus—spreading purple.
Outer glume—purple.
Kernel—white.
Internode—purple on gold.
Grain size II. *c.* Slender . . . Type 103.
Inner glume—tip and base dark gold.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—gold.
Grain size II. *b.* Bold . . . . Type 104.

Starchy rice.
Single grained.
Short outer glume.
Solitary spikelets.
Short internode.

Inner glume—piebald on dark gold.
Apiculus—colourless.
Outer glume—white.
Kernel—white.
Internode—green.
Grain size II. *b*. Bold . . . . Type 105.

Inner glume—brown.
Apiculus—brown.
Outer glume—brown.
Kernel—white.
Internode—green.
Grain size I. *a*. Slender . . . Type 106.
Slender . . . Type 107 Earlier than Type 106.
Bold . . . Type 108.
b—Medium . . . Type 109.
II. *b*. Medium . . . Type 110.
Bold . . . . Type 111 Bearded.
Bold . . . . Type 112 Beardless.

Inner glume—purple—before flowering.
Apiculus—spreading purple.
Outer glume—purple.
Kernel—white.
Internode—green.
Grain size I. *a*. Slender . . . Type 113.
II. *b*. Slender . . . Type 114.
Medium . . . Type 115.

Inner glume—purple—after flowering.
Apiculus—spreading purple.
Outer glume—purple.
Kernel—white.
Internode—purple.
Grain size III. *c*. Bold . . . . Type 116.

Inner glume—purple—at maturity.
Apiculus—spreading purple.
Outer glume—purple.
Kernel—white.
Internode—purple.
Grain size I. *a*. Slender . . . Type 117.
b. Slender . . . Type 118.
II. *b*. Medium . . . Type 119.

Kernel—red.
Internode—purple.
Grain size I. *a*. Slender . . . . Type 120.

Starchy rice.
Single grained.
Short outer glume.
Solitary spikelets.
Short internode.
Inner glume—ripening black.
Apiculus—spreading purple.
Outer glume—purple.
Kernel—dull white.
Internode—green.
Grain size II. *b*. Medium . . . Type 121.
Bold . . . . Type 122.
Apiculus—purple.
Outer glume—white.
Kernel—dull white.
Internode—gold.
Grain size II. *b*. Bold . . . . Type 123

V. Summary.

Winter rice is the most important crop in Bihar and Orissa and this paper deals with the classification of a collection of winter rices (and a few autumn rices) made in 1928.

The classifications of previous workers such as Kikkawa, Graham, Beale, etc., are briefly reviewed. Most of them have based their main divisions on the characters of the grain *i.e.*, its size and shape. But a truly satisfactory classification, that is, one that is both natural and simple, has not yet been forthcoming. In view of this the various plant characters have been considered in detail and their merits for use in classification discussed. It is pointed out that little is known of physiological characters, while most quantitative characters are difficult to use as it is generally impossible to define the classes sharply, and moreover they are markedly subject to fluctuation through changes in the environment. It has therefore been concluded that the qualitative characters are, on the whole, the most suitable for the purpose. The main divisions are based on the chemical constitution of the rice kernel, presence or absence of clustering in spikelets, presence or absence of double-grained spikelets, length of the outer glume, and upon the presence or absence of elongated internods with the production of aerial roots at the nodes. After this the colour of the inner glume has been chosen as the most suitable criterion. The inner glume exhibits a wide range of easily distinguishable colours and affords a ready and convenient means of discrimination.

Next to the colour of the inner glume that of the apiculus is most important as a criterion and two distinct types of colour in this character have been discovered. In the first type the colour is restricted to the extreme tip while in the second type

designated as " spreading apiculus " the colour extends to a part of the inner glume and palea. After the apiculus, the colour of the outer glumes is used. Thereafter the colour of the kernel and internode and finally the grain size and shape which are the most constant among the quantitative characters, are used in separating the ultimate classes. The other characters discussed have been rejected as unsuitable either because they are subject to marked fluctuations, or not easily definable, or because there is a strong correlation between them and one of the characters already employed in the classification.

A key to the classification is provided.

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THE DEVELOPMENT OF PIGMENTS IN THE GLUMES AND APICULUS OF RICE VARIETIES.

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(With Plates XXXVII and XXXVIII)

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I. INTRODUCTION.

The presence of colours other than green in various parts of the plant has been found useful in discriminating between the varieties of paddy. A closer study of the development of the several colours in the important organs used in classification is therefore a necessity. Though genetic behaviour indicates to a great extent the relation subsisting between one variety and another, the study of the course of development of the colours also helps to throw light on taxonomic problems. For example, in the purple inner glume colour group in rice, though the final colour in many varieties appears to be the same, detailed developmental study of the pigment revealed that the group is really composed of three sub-groups—

(i) in which the purple appears before flowering,
(ii) after flowering,
(iii) late in maturity.

It will thus be seen that the periods of flowering and fertilisation greatly influence the colour development.

PLATE XXXVII.

STAGES IN THE DEVELOPMENT OF GLUMES.


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Figs.	1-2	Type	44.—Inner glume.—	Straw colour.
	3-6	,,	76. ,,	Light olive furrowed.
	7-10	,,	87. ,,	Piebald olive on light gold background.
	11-14	,,	89. ,,	Light gold.
	15-18	,,	105 ,,	Piebald on dark gold.
	19-21	,,	100. ,,	Dark gold.

In connection with an attempt to classify the rices of Bihar and Orissa [Kashi Ram and Sarvayya Chetty, 1934], the development of colour in the glumes of the spikelet was followed, as this character of the rice plant affords a greater range and variety of colour than any other part of the rice plant. Further the colour development in these organs extends over a sufficiently long period (about 40 days) to make accurate observation possible.

The course of development of the following colours was traced from the very first appearance of colour, while the panicle was still in boot, to the final mature condition of the grain. The nomenclature adopted has been explained [Kashi Ram and Sarvayya Chetty, 1934] 1. Straw. 2. Ripening straw. 3. Ripening gold. 4. Olive. 5. Gold. 6. Brown. 7. Purple. 8. Ripening black.

Most of the terms used are in confirmity with those of previous workers on the inheritance of characters ; the new terms used are self-explanatory. The date of commencement of blooming is important and is referred to hereafter as the day of flowering.

The colour development in the apiculus has also been followed.

II. Course of development of colours in the spikelet.

(*a*) *Inner glume.*

(*1*) *Straw.*—The colour of the mature inner glume is similar to that of the dry straw. This colour is neither absolutely white nor yellow but a blend of both.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Type 44 :—		
1st stage—		
Green—		
Commencing from the day of flowering	16	XXXVII, Fig. 1.
2nd stage—		
Straw—		
Disappearance of green resulting in the production of "straw" colour as the grain ripens.		
Commencing from the completion of the 1st stage . . .	11	XXXVII, Fig. 2.

(*2*) *Ripening straw.**—Though the inner glumes look on maturity like those of the straw group, the history of development of colour in this group reveals the

* As the several stages in ripening straw, ripening gold, light olive uniform, dark olive furrowed and purple at maturity cannot be correctly reproduced in print, paintings of these were not attempted.

existence of a transient stage characterised by the production of olive-colour specks which fade away later.

	Number of days for the colour to pass from the top to the bottom of the ear.
Type 50 :—	
1st stage—	
Green—	
Commencing from the day of flowering	3
2nd stage—	
Light olive specks—	
Commencing 2 days after flowering	5
3rd stage—	
Light yellow—	
Commencing after the completion of the 2nd stage	3
4th stage—	
Olive specks disappearing and the light yellow becoming lighter due to maturity—	
Commencing from the completion of the 3rd stage	7
5th stage—	
Ripening to straw—	
Commencing after the completion of the 4th stage	7

(3) *Ripening gold.*—Light lemon yellow colour develops over the glume as the ear approaches maturity ; it then fades to straw except for some specks of faded yellow remaining in the furrows near the apex.

	Number of days for the colour to pass from the top to the bottom of the ear.
Type 47 :—	
1st stage—	
Green—	
Commencing from the day of flowering	6
2nd stage—	
Light lemon yellow—	
Commencing from the completion of the 1st stage	3
3rd stage—	
Yellow—	
Commencing 2 days after the completion of the 2nd stage	4
4th stage—	
Light ripening gold—	
Commencing from the completion of the 3rd stage	3
5th stage—	
*Ripening gold fading to straw—	
Commencing a day after the completion of the 4th stage	8

* In all cases the final stage is characterised by the disappearance of the green colour.

(4) *Olive.*—In this group brownish specks appear after flowering, the colour later intensifying to different shades of cold brown. In some types the colour is distributed uniformly over the ridges and furrows—and this is called "uniform" while the appearance of colour in the furrows only is described as "furrowed". "Piebald" denotes the localisation of olive colour to the middle of the glume leaving the top and bottom straw.

Light olive uniform.	Number of days for the colour to pass from the top to the bottom of the ear.
Type 70—	
1st stage—	
Light yellow on light green background—	
Commencing a day before flowering	5
2nd stage—	
Light olive specks—	
Commencing a day after flowering	8
3rd stage—	
Olive specks intensifying in colour and spreading uniformly all over the glume—	
Commencing 4 days after the beginning of the 2nd stage	6
4th stage—	
The green background becoming lighter—	
Commencing 1 day after the completion of the 3rd stage	6
5th stage—	
Light olive uniform changing to woody brown—	
Commencing from the completion of the 4th stage	13

Light olive furrowed.	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Type 76—		
1st stage—		
Light yellow on light green background—		
Commencing 2 days before the day of flowering	5	XXXVII, Fig. 3.
2nd stage—		
Light olive specks in furrows—		
Commencing a day after flowering	7	XXXVII, Fig. 4.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
3rd stage—		
The background becoming lighter with intensification of the olive specks—		
Commencing 2 days after the completion of the 2nd stage . .	6	XXXVII, Fig. 5.
4th stage—		
Light olive furrows changing to woody brown—		
Commencing from the completion of the 3rd stage . . .	14	XXXVII, Fig. 6.
Dark olive furrowed.		
Type 86—		
1st stage—		
Light yellow on light green background—		
Commencing 3 days before flowering	7	...
2nd stage—		
Light olive specks in furrows—		
Commencing from the day of flowering	8	...
3rd stage—		
Olive in furrows—		
Commencing 2 days after the beginning of the 2nd stage . .	8	...
4th stage—		
Dark olive in furrows—		
Commencing a day after the beginning of the 3rd stage . .	8	...
5th stage—		
Green background becoming lighter—		
Commencing from the completion of the 4th stage . . .	10	..
6th stage—		
Dark olive furrows changing to chocolate brown—		
Commencing 5 days after the completion of the 5th stage . .	5	..
Piebald olive on light gold background.		
Type 87—		
1st stage—		
Light yellow on light green background—		
Commencing from the day of flowering	4	XXXVII, fig. 7.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
2nd stage—		
Light olive specks in furrows—		
Commencing from the day of the flowering	8	XXXVII, fig. 8
3rd stage—		
Olive in furrows, becoming prominent in the middle of the glume leaving the top and bottom free—		
Commencing 3 days before the completion of the 2nd stage . .	10	XXXVII, fig. 9.
4th stage—		
Green background becoming lighter—		
Commencing a day after the completion of the 3rd stage . .	4	..
5th stage—		
Olive changed to chocolate brown; as the green background is replaced by light gold the piebald nature becomes more prominent—		
Commencing from the completion of the 4th stage . . .	12	XXXVII, fig. 10.

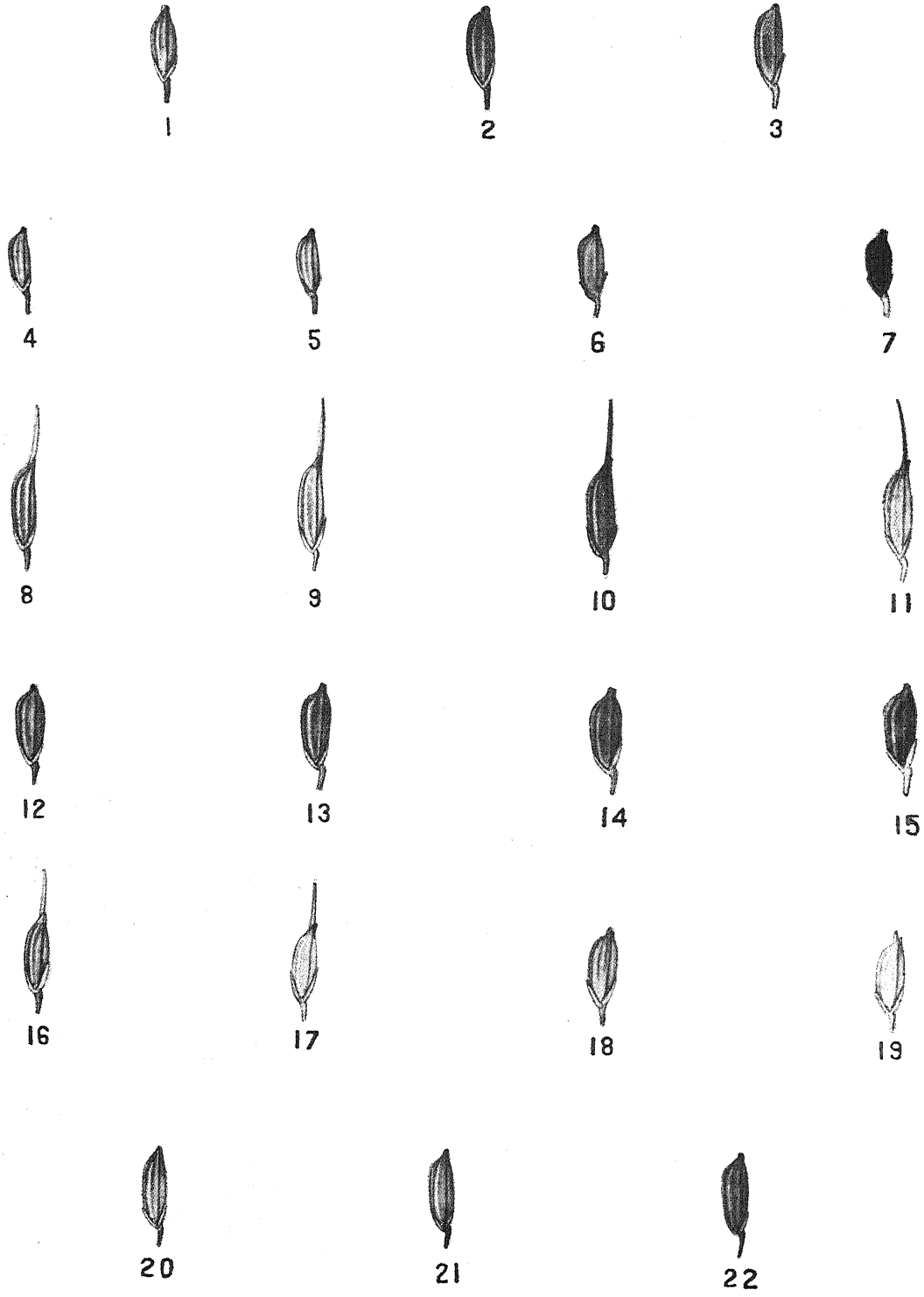
(5) *Gold.*—This group is characterised by the development of reddish coppery colour on a background of rich lemon yellow, the latter being developed a few days before flowering when the ear is still in the boot.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Light gold.		
Type 89—		
1st stage—		
Light yellow on light green background—		
Commencing a day previous to flowering	4	XXXVII, fig. 11.
2nd stage—		
Light coppery red tinge—		
Commencing a day before the completion of the 1st stage . .	5	XXXVII, fig. 12.
3rd stage—		
Light golden yellow—		
Commencing a day before the completion of the 2nd stage . .	8	XXXVII, fig. 13

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
4th stage—		
Green background becoming lighter—		
Commencing a day after the completion of the 3rd stage . .	3	..
5th stage—		
Light gold becoming clear—		
Commencing after the completion of the 4th stage . . .	14	XXXVII, fig. 14.
Dark gold.		
Type 100—		
1st stage—		
Light yellow on light green background—		
Commencing a day before flowering	4	XXXVII, fig. 19.
2nd stage—		
Light coppery red tinge—		
Commencing a day before flowering	6	XXXVII, fig. 20.
3rd stage—		
Gold—		
Commencing from the completion of the 2nd stage . . .	9	..
4th stage—		
The green background becoming lighter—		
Commencing 3 days after the completion of the 3rd stage . .	11	..
5th stage—		
Dark gold becoming prominent—		
Commencing from the completion of the 4th stage . . .	5	XXXVII, fig. 21.
Piebald on dark gold.		
Type 105—		
1st stage—		
Light yellow on light green background—		
Commencing from the day of flowering	3	XXXVII, fig. 15.
2nd stage—		
Coppery red tinge—		
Commencing a day after flowering	7	XXXVII, fig. 16.

PLATE XXXVIII.

STAGES IN THE DEVELOPMENT OF GLUMES AND APICULUS.



Figs. 1-3 Type 109. Inner glume.—Brown.
4-7 ,, 113. ,, Purple before flowering.
8-11 ,, 116. ,, ,, after ,,
12-15 ,, 5. ,, Ripening black on gold background.
16-17 ,, 54. Apiculus.—Spreading brown.
18-19 ,, 58. ,, Localised purple.
20-22 ,, 67. ,, Spreading purple.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
3rd stage—		
Gold becoming prominent in the middle of the glume leaving about ½ of the glume at the tip and base free—		
Commencing a day after the beginning of the 2nd stage . .	3	XXXVII, fig. 17.
4th stage—		
Green background becoming lighter—		
Commencing 12 days after the completion of the 3rd stage . .	9	--
5th stage—		
Piebald gold becoming prominent—		
Commencing from the completion of the 4th stage . . .	7	XXXVII, fig. 18.

(6) *Brown.*—In this group brown pigment develops over an initial lemon yellow colour resulting in a reddish brown tint, when the grain attains maturity.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Type 109—		
1st stage—		
Green—		
Commencing from the day of flowering	5	XXXVIII, fig. 1.
2nd stage—		
Thick olive specks on light yellow background—		
Commencing 4 days after the day of flowering	8	XXXVIII, fig. 2.
3rd stage—		
Light brown—		
Commencing a day after the completion of the 2nd stage . .	13	..
4th stage—		
Brown—		
Commencing a day after the completion of the 3rd stage . .	6	..
5th stage—		
Brown becoming dull—		
Commencing a day after the beginning of the 4th stage . .	10	XXXVIII, fig. 3.

(7) *Purple*—This group is further divided into three sub-groups according as the colour develops before or after flowering or at maturity.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Purple before flowering.		
Type 113—		
1st stage—		
Light yellow on light green background—		
Commencing 4 days before the day of flowering	5	..
2nd stage—		
Purple wash—		
Commencing a day before the day of flowering	6	XXXVIII, figs. 4, 5, 6.
3rd stage—		
Deep purple—		
Commencing 2 days before the completion of the 2nd stage	9	..
4th stage—		
Dark purple—		
Commencing after the completion of the 3rd stage	12	XXXVIII, fig. 7.
5th stage—		
Dull dark purple on fading—		
Commencing at the completion of the 4th stage	6	..

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Purple after flowering.		
Type 116—		
1st stage—		
Green—		
Commencing on the day of flowering	1	..
2nd stage—		
Purple wash—		
Commencing a day after flowering	5	XXXVIII, figs. 8, 9.
3rd stage—		
Purple—		
Commencing 5 days after the completion of the 2nd stage	6	XXXVIII, fig. 10.
4th stage—		
Ripening to straw with purple patches—		
Commencing 6 days after the completion of the 3rd stage	8	XXXVIII, fig. 11.

	Number of days for the colour to pass from the top to the bottom of the ear.
Purple at maturity (on a ripening straw background).	
Type 117—	
1st stage—	
Light yellow on light green background—	
Commencing 4 days before flowering	6
2nd stage—	
Olive specks—	
Commencing 4 days after the day of flowering	4
3rd stage—	
Olive specks disappearing—	
Commencing 7 days after the completion of the 2nd stage	5
4th stage—	
Light purple wash—	
Commencing from the completion of the 3rd stage	4
5th stage—	
Maturing to purple specks on a ripening straw background—	
Commencing a day after the completion of the 4th stage	14

(*8*) *Ripening black.*—This group is characterised by the development of the black colour as the grain attains maturity. The colour begins as a smoky coat and finally fades. This may occur on a background of "straw", "ripening straw", "olive", "gold", or "purple", but only the development over a gold background has been studied.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Ripening black on gold background.		
Type 5—		
1st stage—		
Light yellow on light green background—		
Commencing 3 days before flowering	3	..
2nd stage—		
Yellow—		
Commencing on the day of flowering	4	XXXVIII, fig. 12.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
3rd stage—		
Coppery red tinge—		
Commencing a day after the beginning of the 2nd stage . .	9	XXXVIII, fig. 13.
4th stage—		
Ripening black—		
Commencing 8 days after the completion of the 3rd stage . .	15	XXXVIII, fig. 14.
5th stage—		
Fading to smoky grey—		
Commencing 9 days after the beginning of the 4th stage . .	11	XXXVIII, fig. 15.

(*b*) *Outer glume.*

This organ is usually non-pigmented, small, stiff and shiny. Only two colours—brown and purple—occur. Both these colours fade to lighter shades at maturity.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Brown.		
Type 54—		
1st stage—		
Yellow—		
Commencing 12 days after flowering	5	..
2nd stage—		
Brown—		
Commencing from the completion of the 1st stage . . .	2	..
3rd stage—		
Brown fading—		
Commencing 7 days after the completion of the 2nd stage . .	4	..
Purple.		
1st stage—		
Light purple—		
Commencing 3 days before flowering	6	XXXVIII, Fig. 9.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
2nd stage—		
Purple—		
Commencing from the completion of the 1st stage . . .	7	XXXVIII, fig. 10.
3rd stage—		
Fading purple—		
Commencing 10 days after the completion of the 2nd stage . .	7	XXXVIII, fig. 11.

(c) *Apiculus.*

The apiculus is generally colourless. But two colours—brown and purple—may occur. These are further divided into two main groups—

(*i*) localised or restricted ;

(*ii*) spreading, which is invariably associated with a similar colour in the outer glume.

The course of the development of the brown and purple colour in the apiculus is the same in the several groups.

Brown.—In the brown group, in our collection the localised form is rare hence the apiculus with spreading brown colour has been studied.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Spreading brown.		
Type 54—		
1st stage—		
Yellow—		
Commencing a day after flowering	4	XXXVIII, fig. 16.
2nd stage—		
Deep yellow—		
Commencing a day after the beginning of the 1st stage . .	7	..
3rd stage—		
Light brown—		
Commencing 5 days after the completion of the 2nd stage . .	4	..
4th stage—		
Spreading brown becoming clear, with the disappearance of the green colour in the glume—		
Commencing 3 days after the beginning of the 3rd stage . .	9	..
5th stage—		
Dull brown on fading—		
Commencing at about the completion of the 4th stage . . .	4	XXXVIII, fig. 17.

	Number of days for the colour to pass from the top to the bottom of the ear.	Plate Number.
Purple.		
(i) Localised or restricted purple.		
Type 58—		
1st stage—		
Light purple—		
Commencing 3 days before the day of flowering	3	XXXVIII, fig. 18.
2nd stage—		
Purple—		
Commencing 2 days after flowering	5	..
3rd stage—		
Purple fading—		
Commencing 15 days after the completion of the 2nd stage . .	4	XXXVIII, fig. 19.
(ii) Spreading purple.		
Type 67—		
1st stage—		
Light purple at the extreme tip (= like restricted apicules)—		
Commencing 8 days before flowering	5	XXXVIII, fig. 20.
2nd stage—		
Purple spreading—		
Commencing on the day of flowering	3	XXXVIII, fig. 21.
3rd stage—		
Purple fading—		
Commencing 17 days after the completion of the 2nd stage . .	12	XXXVIII, fig. 22.

III. Summary.

The presence of colour in parts of the rice plant is useful for taxonomic purposes, and the innumerable varieties of rice can be classified on the basis of the presence or absence of the several pigments in the different organs. Of the several parts of the rice plant used in the classification the spikelets is perhaps the most important. On casual observation many varieties appear to be similar but a detailed study of the development of the pigments in the different parts of the spikelet reveals the transient development of colour, *e.g.*, light olive specks in the ripening straw group which would otherwise be indistinguishable from the straw group. Similarly the ripening blacks can be discriminated by the pigments present in the earlier stages.

The detailed study of the growth of the pigments has further revealed the existence of physiologic sub-groups, *e.g.*, in the purple group the development of the pigment is intimately connected with that most important biological character—time of flowering—and on this basis the existence of three different physiologic forms of purple has been indicated. In brief the study has been of value in bringing out small and not easily observed but nevertheless important differences between varieties.

The results show that many pigments commence development after flowering and that in all cases the final stage is characterised by the disappearance of the green (chlorophyll) colour. The several stages noted in the course of development in the case of single 'unmixed' colours, *e.g.*, straw, purple, etc., are not very rigidly defined and are liable to vary with the colour standards of the investigator. But in the case of 'compound' colours the several stages can be more sharply distinguished as each stage is marked by the appearance or the disappearance of a distinct colour.

ACKNOWLEDGMENTS.

The authors desire to acknowledge the assistance which they have received from Dr. B. P. Pal, M.Sc., Ph.D. (Cantab.), Second Economic Botanist, Imperial Institute of Agricultural Research, Pusa, in the preparation of the paper and from Mr. Pushkarnath Thusu, M.Sc. (Hons.), post-graduate student, who has been of great assistance in the field observations.

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THE ADSORPTION AND ELUTION OF CUCUMBER MOSAIC VIRUS†.

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It is generally agreed that the failure of many viruses to pass through various filter candles is partly due to the adsorption of the active agents on the walls of filter pores rather than to their retention due to the size of the respective particles. Since cucumber mosaic virus is not filterable through the Pasteur-Chamberland candles, this may indicate a high adsorptive capacity on the part of this virus. The experiments to be described in this paper were designed to test this possibility.

One of the main difficulties in working with the virus of cucumber mosaic is that the infectious juice cannot be freed of its suspended solid matter. The passage of sap extracted from mosaic cucumber plants through a sand-and-pulp filter or through a bed of kaolin or Fuller's earth removes the virus by adsorption, and the clear brown filtrate is invariably non-infectious. It is evident that, until it is possible to obtain this virus in a clarified state, experiments on its adsorption are not possible with filter candles due to the rapid clogging of their pores by suspended materials in the plant juice containing the virus. The following experiments have therefore been carried out with two particulate substances which were considered likely to adsorb the virus.

MATERIALS AND METHODS.

The infective extract was prepared in the manner previously described by Henderson Smith [1928]. Infected leaves from cucumber plants in active growth were weighed and cut into very small pieces with sterile scissors and ground in a mortar. After the leaves had been reduced to a fine pulp, a small quantity of water was added from time to time and grinding continued, until 300 c.c. of water was added per 100 grms. of tissue. The pulp was sqeezed through muslin and the juice obtained was passed through folded filter paper to remove all suspended materials ; the filtered juice is referred to in this paper as cucumber virus extract.

† This investigation was carried out in the Department of Plant Pathology, Rothamsted Experimental Station, England.

The particulate substances used were Fuller's earth and kaolin. Each adsorbent to be tested was weighed carefully and placed in beakers of 250 c.c. capacity ; appropriate quantities of the cucumber virus extract were then added and the resulting suspension shaken vigorously for 10 minutes. After the mixture had been shaken, it was passed through filter paper and the filtrate obtained was inoculated to cucumber plants. The cucumber virus extract (without adsorbents) meant for inoculating control plants was also shaken for 10 minutes as it seemed possible that the inactivation of the virus in the extract treated with an adsorbent may be hastened by prolonged shaking due to oxidation. The pH of the extract and filtrates was determined by the electrometric method.

Difficulty was experienced at the outset in the inoculation of cucumber plants as the percentage of infection was consistently low, although plants were inoculated, as suggested by Doolittle [1920], after they had produced from 6 to 8 leaves. This necessitated a number of trials to determine the conditions under which a high percentage of infection can be obtained with cucumber mosaic. Excellent results were obtained by rubbing firmly the surface of the cotyledonary leaves, supported on a plant label, with a piece of cotton wool soaked in the virus extract. An attempt was made to break the stiff trichomes without unduly lacerating the cotyledons. The first true leaf was then rubbed with the virus-impregnated cotton wool, supporting the leaf on the left hand. It was necessary to apply gentle friction to produce mechanical injury, since a slight pressure tears the leaf. The second true leaf was also similarly treated. It was found that plants inoculated in this manner always gave 100 per cent. infection, provided the inoculum was obtained from young infected leaves of actively growing plants.

All the apparatus was previously sterilised, and inoculations were made within 6 to 8 hours from the time the infected leaves were removed from the plant. In all cases inoculated plants were held under observation for a period of not less than a fortnight. Symptoms were usually visible 6 or 7 days after inoculation, and in a few cases they were delayed in appearing by further 2 or 3 days. After each treatment the hands were always washed thoroughly with soap.

Experiments on the clarification of the virus extract.

In preliminary experiments the cucumber virus extract was passed through a sand-and-pulp filter or through a bed of Fuller's earth made by passing through filter paper a suspension containing 2·5 grms. of Fuller's earth in 100 c.c. of distilled water. Tobacco and cucumber plants were inoculated with the filtrates, which proved non-infectious. The control cucumber plants were all infected (Table I).

TABLE I.

Results of inoculation to cucumber plants with clarified filtrates.

Filtrates	pH		Cucumber		Tobacco	
	Virus extract	Filtrate	Inoculated	Infected	Inoculated	Infected
Sand-and-pulp filter . .	7·7	6·5	5	0	5	0
Sand-and-pulp filter . .	7·6	6·4	4	0	..	..
Fuller's earth bed . .	7·7	6·5	5	0	5	0
Virus extract . . .	7·7	..	2	2	..	..
Virus extract . . .	7·6	..	2	2	..	..

EXPERIMENTS ON THE ADSORPTION OF CUCUMBER VIRUS.

In these experiments kaolin and Fuller's earth were used to adsorb cucumber mosaic virus. Quantities of adsorbents ranging from 0·025 to 1·25 grms. were suspended in 100 c. c. virus extract ; the resulting mixtures were shaken for 10 minutes and passed through filter paper yielding clear brown filtrates. The pH of each filtrate was determined before inoculation. In each series suitable controls were set up. The results of two representative experiments are recorded in Table II.

TABLE II.

Results of inoculation to cucumber plants with virus extract treated with adsorbents.

Filtrates (in 100 c.c. extracts)	pH		Cucumber		Control	
	Virus extract	Filtrate	Inoculated	Infected	Inoculated	Infected
Kaolin—						
1·25 grms. . . .	8·3	8·1	2	0	2	2
1·25 grms. . . .	7·6	7·8	3	0	2	2
0·625 grm. . . .	8·3	8·1	3	0	2	2
0·625 „ . . .	7·6	7·8	3	0	2	2
0·125 „ . . .	8·4	8·3	4	0	4	4
0·125 „ . . .	7·6	7·8	3	0	2	2
0·025 „ . . .	8·4	8·2	4	2	4	4
0·025 „ . . .	7·6	7·8	3	3	2	2
Fuller's earth—						
1·25 grms. . . .	8·3	8·0	2	0	2	2
0·625 grm. . . .	8·3	8·1	3	0	2	2
0·625 „ . . .	8·4	8·2	3	0	4	4
0·125 „ . . .	8·4	8·2	4	0	4	4
0·025 „ . . .	8·4	8·3	4	2	4	4
0·025 „ . . .	7·6	7·7	3	3	2	2

It will be seen from Table II that both kaolin and Fuller's earth possess unusual powers of adsorption when mixed with the cucumber virus extract. The adsorbents removed the virus from the juice even when used in concentrations as low as 0·125 grm. in 100 c.c. of the extract. These results may be expected considering that the virus does not pass through various grades of the Pasteur-Chamberland candles.

Experiments on the effect of the pH of the extract on the infectivity of the virus.

The cucumber virus extract was distributed in 20 c.c. volumes in five beakers, to which definite quantities of $N/10$ HCl were added in increasing amounts. Each beaker was shaken for about 2 minutes and the pH of the extract determined electrometrically. The extracts were inoculated to cucumber plants in active growth. The results are summarised in Table III.

Table III.

Effect of pH on the infectivity of the virus.

pH	Cucumber	
	Inoculated	Infected
7·8 (Virus extract)	3	3
6·5	4	4
5·3	4	3
4·5	4	0
3·5	4	0
2·5	4	0

It will be seen that cucumber virus is readily inactivated in hydrogen-ion concentrations greater than pH 5, and, as will be shown later, there are indications that, in more alkaline media than pH 9, it is also rendered non-infectious. Unlike the virus of ordinary tobacco mosaic [Fukushi, 1933], cucumber mosaic virus is active only within a rather narrow range of pH, *i.e.* 5-9.

Experiments on the elution of cucumber mosaic virus.

In view of the fact that very small quantities of kaolin or Fuller's earth are capable of adsorbing cucumber virus, it was of interest to determine whether adsorp-

tion results in the inactivation of the virus, or whether it is possible to free the virus in a state in which it is again capable of producing the disease. The method employed to elute the virus was by changing the pH of the virus extract to the alkaline or acid side with ammonia solution or *N*/10 HCl.

It is not necessary to describe in detail the different experiments; but the following method was generally used. Fuller's earth was employed as the adsorbent in concentrations of 0·625 or one grm. in 100 c.c. virus extract. The virus-adsorbent mixture was shaken vigorously for 10 minutes and passed through filter paper. The residue was re-suspended in 33 c.c. of distilled water, and the pH of the mixture was changed to the acid side with the addition of *N*/10 HCl. The mixture was again shaken for 10 minutes and allowed to stand for 15-20 minutes; the supernatant liquid was decanted and inoculated to cucumber plants. In the other case the residue on the filter paper was suspended in 33 c.c. of 0·04 and 0·08 per cent. ammonia solution, and the mixture was shaken for 10 minutes and allowed to stand for at least half an hour. The supernatant liquid was decanted in the usual manner, and in some cases it was used as such to inoculate plants, whilst in others its reaction was adjusted to pH 5-7 before inoculation. Suitable controls were always made with the virus extract and with the filtrate of the extract treated with the adsorbent.

TABLE IV.

Experiments on the elution of cucumber virus adsorbed to Fuller's earth.

Treatment	Weight of adsorbent in 100 c. c. extract	Cucumber	
		Inoculated	Infected
	Grms.		
Suspended residue adjusted to pH 6·7	0·625	3	2
Suspended residue adjusted to pH 6·6	1·00	3	2
Suspended residue adjusted to pH 6·2	1·00	3	1
Suspended residue adjusted to pH 6·0	1·00	3	1
Suspended residue adjusted to pH 5·7	0·625	3	0
Suspended residue adjusted to pH 5·2	1·00	3	0
Residue treated with 0·1 per cent. ammonia solution	0·625	3	0
Residue treated with 0·08 per cent. ammonia solution	1·00	3	0
Residue treated with 0·08 per cent. ammonia solution, but reaction adjusted to pH 6·7.	1·00	3	1
Residue treated with 0·04 per cent. ammonia solution	1·00	3	0
Residue treated with 0·04 per cent. ammonia solution, but reaction adjusted to pH 6·7.	1·00	3	3

Controls always gave 100 per cent. infection, and the filtrate of the extract treated with the adsorbent was non-infectious.

From the results in Table IV, it will be seen that cucumber mosaic virus adsorbed to Fuller's earth can be freed *in vitro* in an active state by altering the pH of the medium to 6-6·7. If, however, the medium is more acid than pH 6, no infection is obtained, due probably to the inactivation of the virus. When the residue is treated with an ammonia solution, the supernatant liquid is non-infectious; but it is evident that the virus has not been irreversibly inactivated since it is possible to release it in an active state by changing the pH to the acid side, *i.e.*, pH 6·7. It seems, therefore, that the adsorption and inactivation of cucumber mosaic virus are reversible phenomena.

As shown above, the supernatant liquid, when adjusted to pH 6-6·7, produced the disease on inoculation to cucumber plants. It was, therefore, of interest to ascertain whether there were any virus particles still adsorbed to the sediment, which have not been freed *in vitro*, but which may be released *in vivo* when the sediment is rubbed on cucumber leaves. To test this point the following experiment was made.

Forty-five c.c. of the virus extract were shaken for 10 minutes with 0·450 grm. of Fuller's earth, and the mixture was passed through filter paper. The residue was re-suspended in 15 c.c. of distilled water, the reaction of the suspension being adjusted to pH 6·2. The suspension was again shaken for 10 minutes and the supernatant liquid decanted in the usual manner. The sediment was then suspended in 5 c.c. of distilled water and tested for the virus. It was found that, although the supernatant liquid was infectious, its sediment, when suspended in distilled water and rubbed on leaves of cucumber plants, failed to produce the disease.

Summary.

The passage of the cucumber virus extract through a sand-and-pulp filter or a bed of Fuller's earth renders the filtrate non-infectious due to adsorption of the virus.

Cucumber mosaic virus is readily adsorbed when such small quantities as 0·125 grm. of kaolin or Fuller's earth are added to 100 c.c. of the virus extract.

Adsorption does not necessarily inactivate the virus unless the hydrogen-ion concentration of the medium is greater than about pH 5.

Cucumber mosaic virus is active only within a rather narrow range of pH, *i.e.*, about 5-9, since hydrogen-ion concentrations greater than pH 5 inactivate the virus. There are indications that the virus is rendered non-infectious in alkaline reactions of more than pH 9.

The virus can be eluted by changing the pH of the suspension to the acid side, *i.e.*, pH 6-6·7. Elution is not possible with an ammonia solution, which, however,

does not inactivate the virus since a change in the reaction of the suspension treated with ammonia to pH 6·7 frees the virus *in vitro* in an active state.

The work was carried out under the auspices of the Imperial Council of Agricultural Research, to which the writer takes pleasure in expressing his sincere thanks for financial assistance. He is also indebted to Sir E. J. Russell for the laboratory and greenhouse facilities at the Rothamsted Experimental Station, and to Dr. J. Henderson Smith who suggested the problem.

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STEM ROT OF TOBACCO CAUSED BY *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY.

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(With Plates XXXIX-XLII)

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I. Introduction.

In 1931-32 there were 1,141,000 acres under tobacco in British India. Of this Bengal contributed nearly a fourth, the major part of which is in the Rangpur District where the light soil, climate and water supply are favourable for its cultivation.

In March 1932, a serious disease causing a rot of the stems of tobacco became manifest on the Tobacco Farm at Rangpur. Whether the disease existed in the crop for a long time and assumed serious proportions that year or whether it came with some imported seed, is not possible to say.

II. Symptoms of the disease and causal organism.

The first manifestation of the disease is a lesion on the stem at the ground level. The stem becomes lighter brown in colour at that place and assumes a water-soaked appearance and within a short time the leaves droop and the plant wilts. Field observations show that the infection is caused by some fungus inhabiting the soil for the lesion has never been seen at any other place than the ground level.

As the disease advances the affected plants bend over and the surface of the stem becomes chalky white. Examination with a lens reveals the fact that the white colour is due to the mycelium of a fungus. If the affected stems are split open, thick ashy brown threads can easily be seen infesting the internal tissues and in the hollow of the pith, large, longish sclerotia lie embedded. The tissues when examined microscopically show a hyaline mycelium filling the cell cavities and also the intercellular spaces. The middle lamella is destroyed and the tissues look very much disintegrated.

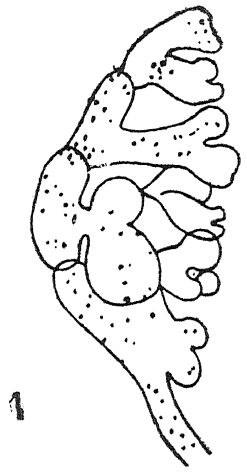
The fungus infesting the tissues can easily be brought into cultivation on culture media. When fragments of the affected stem, surface sterilized with a 0·1 per cent. mercuric chloride solution and then washed with sterilized water, are placed on Quaker Oat agar slants, the fungus hyphae can be seen growing on the surface of the agar in five to six days and in two weeks' time a thick white velvety growth can be seen covering the entire surface of the agar. Black sclerotia are also formed in the mycelium, their surface covered with minute drops of a yellowish liquid. The same fungus develops if sclerotia, after washing with mercuric chloride solution (1 : 1000), dipped in alcohol and flamed, are planted on the agar. The resulting growth is also snowy white and velvety, and sclerotia appear as before. From an examination of this fungus and the sclerotia, it became evident that it belonged to the genus Sclerotinia and that it possibly was *S. sclerotiorum* (Lib.) De Bary.

III. History of the fungus.

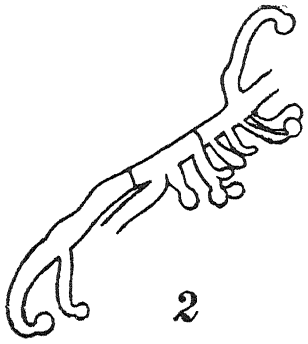
A disease of tobacco due to a Sclerotinia was described by Oudemans and Koning [1905-06] from Java and thinking that it was a new fungus, they had named it as *Sclerotinia nicotianae*. Recently Boning [1933] has shown that the Sclerotinia of Oudemans and Koning was the same as *Sclerotinia sclerotiorum* (Lib.) De Bary.

The fungus *S. sclerotiorum* occurs throughout the world in cooler parts of subtropical and temperate climates and causes a good deal of damage to several

PLATE XXXIX.



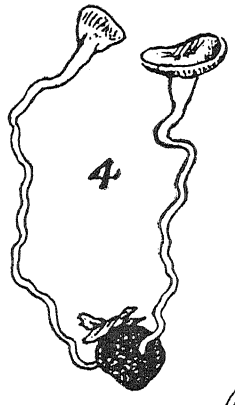
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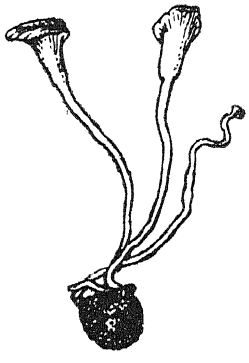
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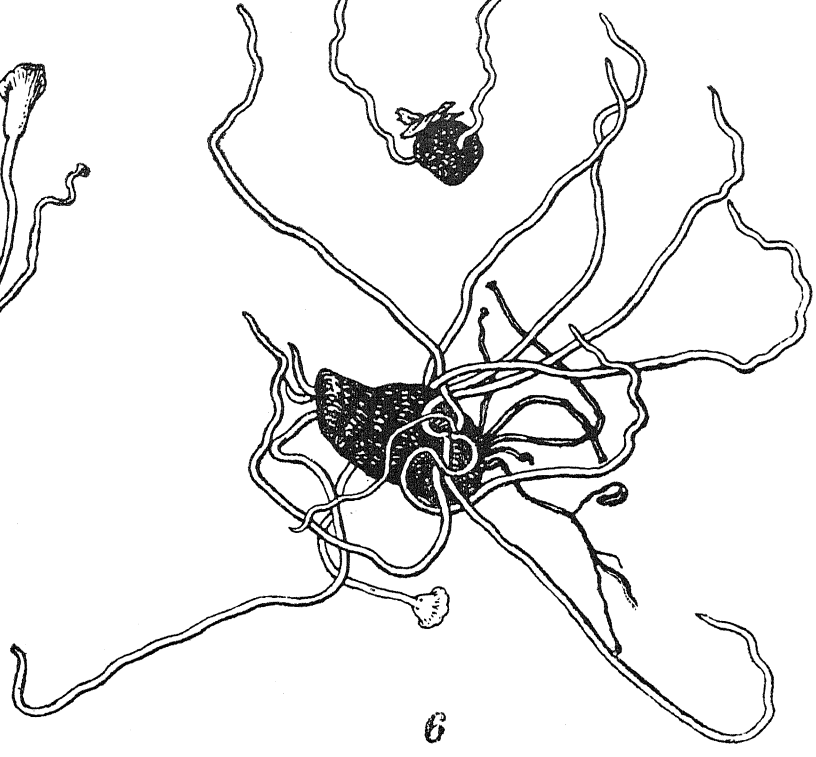
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4



5



6

K. Das.

(For explanation see page 673.)

crops. Smith and Smith [1911] noted that it was very troublesome to lettuce-growing parts in the United States of America and Ramsay [1925] reported it as causing damage to vegetable in transit. In England Dowson [1926] has shown that it causes a disease of the inflorescence and stems of Antirrhinums and in India Joshi [1924-25] found it causing a wilt disease of safflowers. Recently the author has found it attacking *Cannabis sativa* L. in North Bihar.

The host range of *S. sclerotiorum* is very wide and in some years when conditions are favourable, it can cause much damage.

IV. Physiological features of the pathogene.

(*a*) *Growth characters in various culture media.*—The fungus can readily grow in a number of common media and best growth always takes place in those that are rich in organic matter. To note the cultural characters, it was grown in medicine bottles in which the respective media were placed, sterilized and the bottles slanted. The agar media used were Coon's agar, potato dextrose agar, Quaker Oat agar and Kotila's agar. Sclerotia grown in pure cultures on Quaker Oat agar were planted in the centre of the surface. The bottles were placed on a laboratory bench from the 3rd to 7th March 1933. The linear rate of growth was measured on the 4th day. The average diameter of the colony that formed on Coon's agar was 5·2 cm., on potato dextrose agar 9·0 cm., on Quaker Oat agar 6·1 cm. and on Kotila's agar 6·3 cm. Potato dextrose agar seemed therefore to be the best for promoting vegetative growth.

The number of sclerotia that had formed was counted after one month. Quaker Oat agar seemed in this respect to be the best, the average per bottle being seventy-one, while the least number had formed in Coon's agar where the average was twenty-one. On potato dextrose agar and Quaker Oat agar they were produced both on the surface as well as on the edges of the medium while in Coon's and Kotila's agar they were only at the edges. The size of the sclerotia ranged from 0·4 cm. to 1·5 cm.

Radiating strands of the mycelium were very marked on Kotila's and Coon's agar while they were absent on Quaker Oat and potato dextrose agar. Tufted aerial mycelium was very marked on Quaker Oat agar. The aerial hyphal filaments when they touch a solid surface repeatedly branch in a close compact fashion forming organs of attachment (Plate XXXIX, fig. 1).

In the summer, the bottles containing the fungus were placed in the frigidaire at a place where the temperature was about 10°C. When they were examined after four months, it was observed that in potato dextrose and Kotila's agar bottles, the sclerotia had put forth long apothecial processes. The bottles were

then removed and placed on a table in diffuse light in the early part of winter. In three weeks' time the processes formed disc-shaped structures at the ends (Plate XL, fig. 1). In those cultures however that were kept in the dark in the frigidaire for a longer time these processes did not expand into apothecial cups. Light seemed therefore to be an important factor in the development of apothecia.

The number of processes formed by a sclerotium depended upon its size. A sclerotium whose diameter was 0·6 cm. had twenty-six processes most of which later developed into apothecia and another sclerotium that was 0·3 cm. in diameter had only six apothecia. The stalks on which the apothecia are borne are of various length, the longest being 2·2 cm. and the shortest 1·4 cm. In bottles left at room temperatures, the sclerotia did not develop either the processes or the apothecia and it is evident therefore that cooler temperatures than those ordinarily obtaining in the room were necessary to give the required stimulus.

(*b*) *Sclerotia.*—In the formation of the sclerotia the first indication is the appearance of a speck, a minute mycelial knot in the mycelium around which hyphae conglomerate. The speck gradually enlarges, turns chocolate brown and ultimately assumes a black colour. Drops of a clear yellowish liquid appear on the developing sclerotium but as the cultures age, they dry up. The surface of the sclerotium may be covered by a mycelial weft. Sections of the mature sclerotia show a hard black-coloured crust and pith made up of thin-walled pseudo-parenchymatous cells. Air spaces abound and cells are hyaline. The sclerotia do not have any regular shape. As already stated, their size may vary from 4 to 15 mm., the average of fifty being 6 mm. in diameter.

(*c*) *Microconidia.*—As this fungus forms microconidia, it was grown in test-tubes containing potato dextrose agar to see if this particular isolate also produces them. The tubes were examined on alternate days to note their development. There was no sign of any microconidia for nearly a month but after the fifth week microconidia began to develop in some of the tubes when most of the available food material was used up in the process of sclerotial and mycelial formation. It seemed that two per cent. potato agar promoted the best microconidial development (Plate XXXIX, fig. 2). Addition of dextrose seemed to have a deleterious effect. Microconidia also developed abundantly when sclerotia were put into tubes containing sterilized distilled water. They were also seen on mature apothecia (Plate XXXIX, fig. 3) though none were observed developing in platings of single ascospores. Microconidia did not germinate in spite of a large number of attempts. in two per cent agar medium, in sterilized distilled water and rain water they put forth small germ tubes in twenty-four hours but the growth ceased and no changes in temperature or light could induce them to develop further.

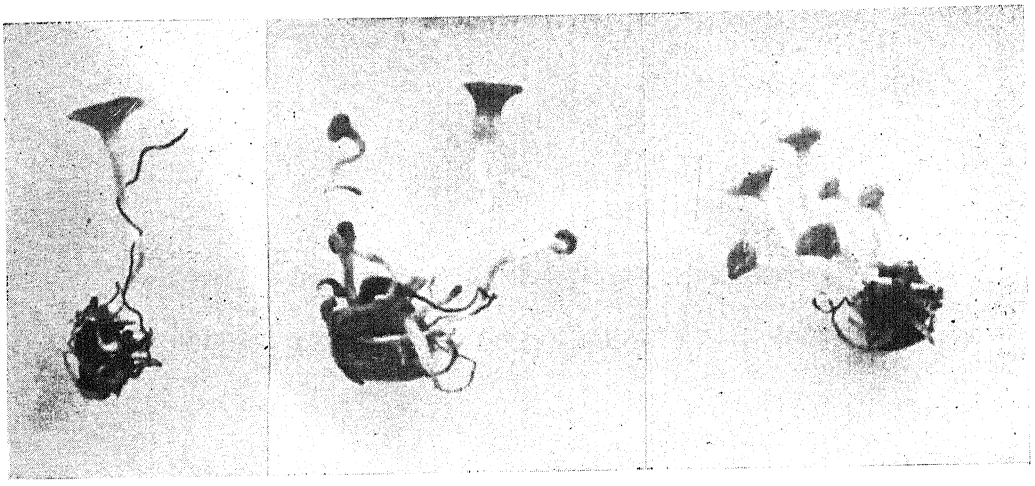
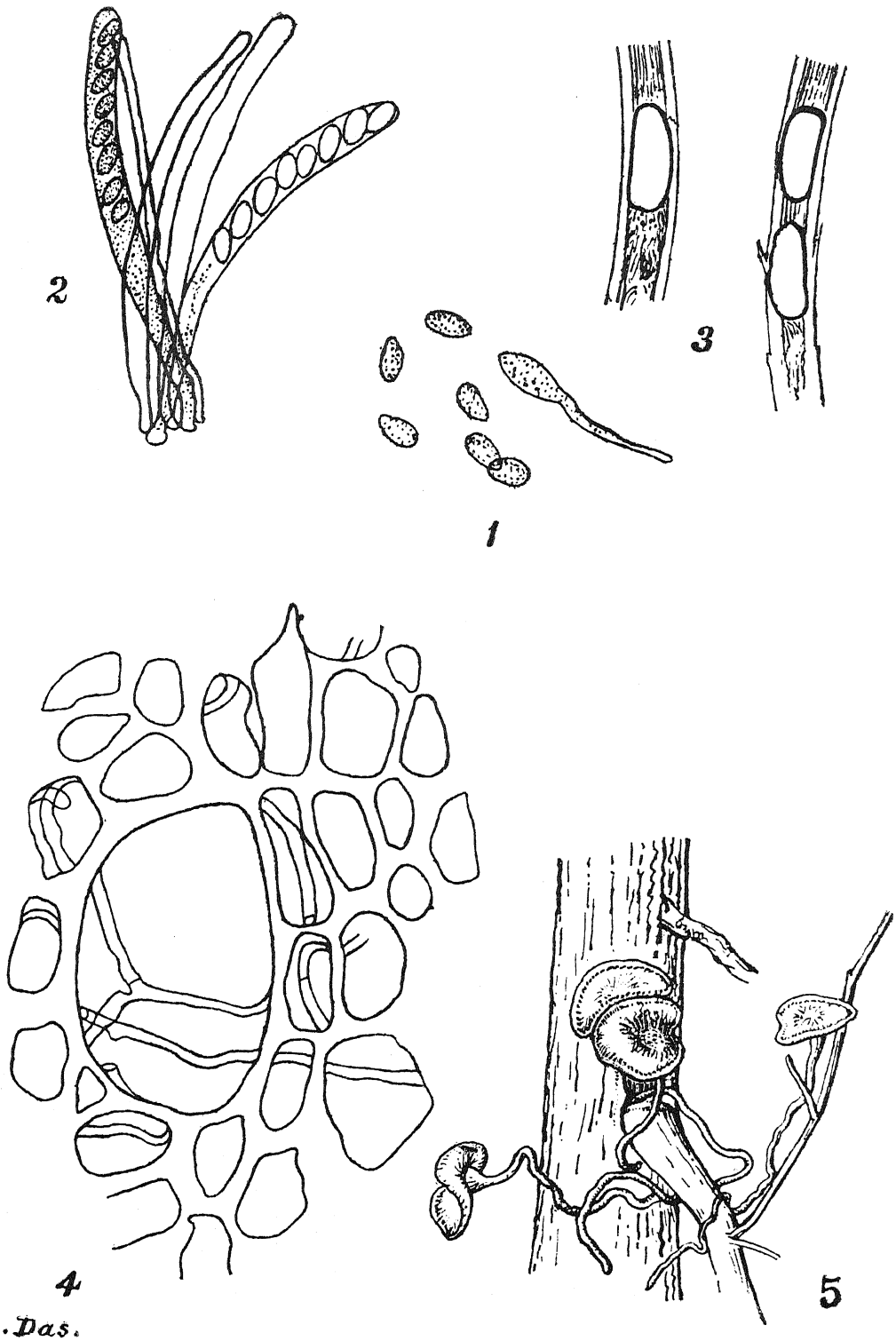


Fig. 1. Apothecia growing from sclerotia in culture (×3).



Fig. 2. Apothecia growing from sclerotia on sawdust (×4).

PLATE XLI.



(For explanation see page 673.)

(*d*) *Apothecia.*—Sclerotia obtained directly from the host and also from cultures were planted in sterilized moist stand, sawdust, and clay soil in Petri dishes and kept at a low temperature in a frigidaire in April 1932. After four months light buff-coloured stalks were observed arising from sclerotia. When the stalks had become about 2·5 cm. long, the Petri dishes were taken out from the cool incubator and kept in alternating light and darkness on a laboratory bench. In a fortnight the tips of apothecia became enlarged forming into small wine-glass shaped fructifications. When the apothecia became fully matured they expanded into discs with striations converging towards the centre (Plate XXXIX, fig. 4). Fifteen stalks were observed on one large sclerotium. The longest stalk was 6 cm. long. The number of sporophores formed depended on the size of a sclerotium. On a small sclerotium three processes were formed (Plate XXXIX, fig. 5) two of which expanded into discs, while on a big sclerotium the processes were fifteen but only one expanded into a disc-shaped structure (Plate XXXIX, fig. 6). There were many sclerotia whose processes failed to expand into cups though the tips became tubular but did not develop. In sawdust the greatest number of sclerotia produced apothecia (Plate XL, fig. 2) and in clay soil the least. A duplicate set of Petri dishes was kept on a laboratory bench throughout but in none of them did the sclerotia develop processes. The result of this experiment also confirms the observations made in the previous one that low temperature induces the development of sporophores. When a Petri dish containing mature apothecia was opened a little puff of spores was observed, due to the forcible discharge of ascospores from mature asci. This is due to the atmospheric disturbance caused by the opening of the lid of the Petri dish. In the hymenial layer, long filiform paraphyses are numerous. The ascus is cylindrical and eight-spored. The ascospores are ovoid or ellipsoidal, some are flattened on one side (Plate XLI, fig. 1). In a good many asci a remnant of protoplasm remains at the base (Plate XLI, fig. 2). The range and average measurements of four hundred asci and ascospores respectively are given in Table I.

TABLE I.

Range and average measurements in microns of asci and ascospores of S. sclerotiorum *of tobacco.*

	Length			Breadth		
——	Range	Mean	Coef. of variation	Range	Mean	Coef. of variation
Asci . . .	81-127	110·26±·45	8·54 per cent	6·8-10·2	6·8±0	*Nil*
Ascospores . .	7·4-15·3	10·79±·043	11·89 per cent	4·4-6·8	6·2±·04	18·4 per cent

(e) *Temperature relation studies.*—Temperature relationships of the fungus were studied on Kotila's agar which is made as follows :—

Potassium dihydrogen phosphate	1·25 grms.
Calcium nitrate	2·36 ,,
Magnesium sulphate	0·59 ,,
Maltose	6·25 ,,
Malt extract	6·25 ,,
Agar	20·00 ,,
Distilled water to make	1,000 c.c.

(Sterilized at 10 lbs. pressure for fifteen minutes.)

For each temperature there were five Petri dishes which had been selected for uniformity. Care was taken to see that the depth of agar in them was uniform. The temperatures available were 10°, 20°, 25°, 30° and 35°C. and the dishes were placed in the respective incubators after inoculating each dish with a single sclerotium. Beginning with the third day the diameters of the colonies that had formed were measured in centimeters at two places in each Petri dish and the readings from the five dishes for each temperature were averaged.

TABLE II.

Growth in centimeters of S. sclerotiorum *at different temperatures in Petri dishes.*

May 19	21	22	23	24	25	26	27
10° . . . . Sown	..	0·9	2·6	4·2	5·6	7·6	8·8
20° . . . . ,,	2·1	5·3	9·1	Growth filled the Petri dishes			
25° . . . . ,,	2·2	5·4	9·1				
30° . . . . ,,	..	..	..				
35° . . . . ,,	..	..	..				

Temperatures ranging from 20° to 25° seemed to favour optimum growth of the fungus. On the fourth day the whole area of the Petri dishes was covered with growth at these temperatures. The fungus did not grow in Petri dishes placed at 30° and 35°C., respectively. After the eighth day, these Petri dishes were placed on a laboratory bench. There was no growth even after fifteen days. These tests were repeatedly done with confirmatory results. In Petri dishes placed at 25°C. the largest number of sclerotia were produced. It may be of interest to mention that the sclerotia formed at 10° and 20°C. developed later apothecia but none were produced in those that were placed at 25°C.

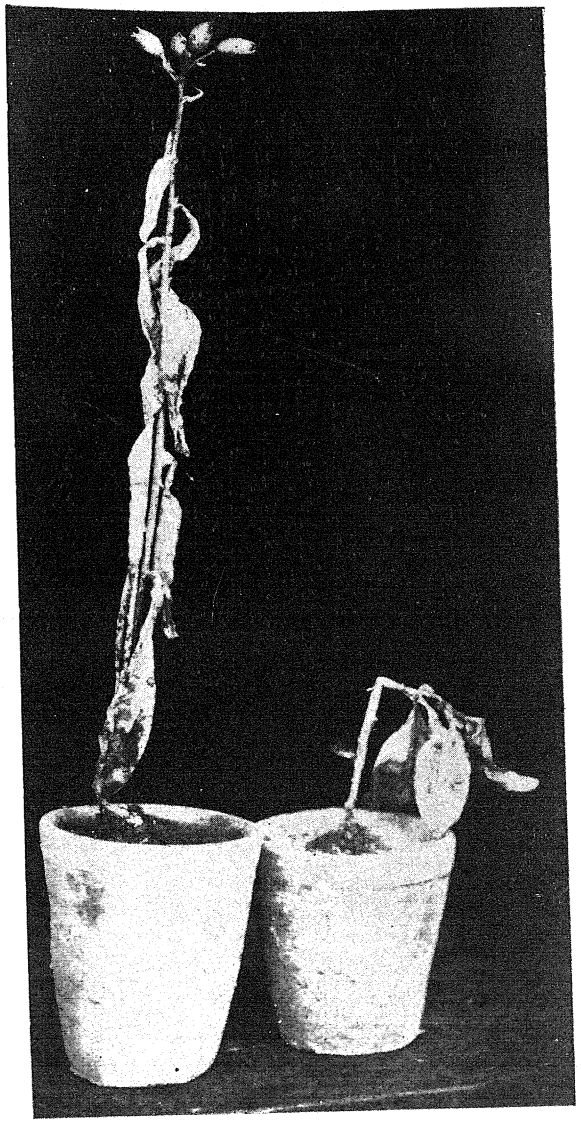


Fig. 1. Artificially infected tobacco plants ($\frac{1}{3}$ actual size).



Fig. 2. Apothecia growing on the surface of an infected soil in a pot ($\frac{1}{2}$ actual size).

V. Pathogenicity.

Infection experiment No. 1.—Inoculations of twenty tobacco seedlings about a foot high were made with pure cultures of the pathogene obtained from single ascospores, by introducing the mycelium and sclerotia into slight incisions made by inserting a sterilized knife into the stems just above ground level. The wounds were wrapped with damp cotton wool which was kept moist. Ten plants served as controls. Three of the inoculated plants showed signs of the disease after a week when the characteristic pallid colour was observed on the stems both above and below the place where the inocula were inserted. The leaves became yellow and then shrivelled and the whole plant hung over (Plate XLII, fig. 1). On splitting open the infected plants many longitudinally elongated sclerotia were found in the hollow of the pith which had been destroyed by the fungus (Plate XLI, fig. 3). Strands of mycelial filaments were also present in the pith cavity. In transverse section of the infected stem, the mycelium was intracellular ramifying in all the tissues (Plate XLI, fig. 4). The control plants remained healthy. The fungus was re-isolated from the infected plants and it agreed with the parent organism in morphological characters.

Table III.

Infection of tobacco plants with mycelium and sclerotia.

Fungus	Where inoculated	Date of inoculation	Number inoculated	Date of infection	Number took infection
Cultures with mycelium and sclerotia.	Wounded stems.	6th February 1933.	20	14th Feb.	3
				16th Feb.	3
				20th Feb.	5
				28th Feb.	4

Infection experiment No. 2.—Cultures containing many sclerotia were mixed with soil in 50 pots and healthy seedlings of tobacco were transplanted in this artificially infested soil in the pots. Twenty pots served as controls and were not mixed with the fungus. Wilting of plants was visible after six weeks with characteristic symptoms. In one pot apothecia were observed at the base of the dead plant (Plate XLI, fig. 5). In Plate XLII, fig. 2, will be found the apothecia growing on the surface of the infected soil, though the plant had not become infected at the time the photograph was taken. Twenty-five plants became infected in the course of three months while the control plants all along remained healthy. The fungus was

re-isolated from the infected plants and it agreed with the parent organism in morphological characters.

Infection experiment No. 3.—Infection experiment was carried out to see whether cut sclerotia are able to bring about infection in healthy plants. The sclerotia were accordingly sterilized in 0·1 per cent. solution of corrosive sublimate for one minute and then washed in several changes of sterilized distilled water. They were then cut into two by a sterilized knife. Inoculations were made on twenty plants by making incisions on the stems at ground level and inserting those cut sclerotial fragments with a small quantity of potato dextrose medium to afford suitable conditions for the sclerotia to germinate. The wounded parts of stems were wrapped with wet cotton wool which was subsequently kept moist. Potato dextrose medium without the fungus was introduced into the incised stems of twenty other plants which were kept as controls. One plant took infection in a week and in the course of twenty days eight plants wilted. The controls remained healthy. The fungus was re-isolated from the infected plants and it agreed with the original organism in all morphological characters.

TABLE IV.

Infection of tobacco plants with cut sclerotia.

Fungus	Where inoculated	Date of inoculation	No. inoculated	Date of infection	No. took infection	Controls
With cut sclerotia . .	Wounded stems.	10th April 1933.	20	17th Apl.	1	5 Controls remained healthy.
				20th Apl.	4	
				27th Apl.	2	
				1st May	1	

From the results given above, it will be seen that the mycelium originating from cut sclerotia is not in any way less parasitic or less viable. Halves of cut sclerotia when planted on potato dextrose agar produced also the usual number of sclerotia after a few days' growth.

Infection experiment No. 4.—Apothecial cups were clipped off with sterilized scissors and placed inverted on wounds made on seven leaves of tobacco in a moist chamber. Drops of sterile water were added just at the base of the cups to keep them moist. Three leaves were kept as controls. In five days three leaves showed light brown discoloration which gradually extended and at last involved the whole surface of leaves. The light brown colour became dark as the infected areas on

the surface of the the leaves extended. The fungus was re-isolated from the diseased tissue and it agreed with the original fungus.

Apothecia were shaken in sterile distilled water in a watch glass. When the sterile water was examined under a microscope there were many ascospores in it but no pieces of mycelium. By means of a sterilized pipette a few drops were placed on the wounded surface of seven healthy tobacco leaves in a moist chamber. Three leaves were kept as controls. Three leaves became infected in three days and one leaf two days afterwards. The controls remained healthy. The fungus was re-isolated from the infected leaves. The experiment was repeated on healthy unwounded leaves and one leaf became infected on the fourth day. The controls remained healthy. The results of these inoculation experiments are summarised below.

TABLE V.

Infection experiments on wounded and unwounded tobacco leaves with ascospores.

Fungus	Where inoculated	Date of inoculation	No. inoculated	Date of infection	No. took infection	Controls
Apothecia .	Wounded leaves	5th Dec. 1933 .	7	8th Dec. 1933 .	2	3
				10th Dec. 1933 .	1	
Ascospores only .	Do.	9th Jan. 1934 .	7	12th Jan. 1934 .	3	3
				14th Jan. 1934 .	1	
	Unwounded leaves.	Do.	8	13th Jan. 1934 .	1	3 All the controls remained healthy.

De Bary [1887] and other investigators could not get the ascospores to infect the host and the former is of the opinion that the promycelium from the ascospores passes some part of its life saprophytically on dead organic matter and then becomes parasitic. This experiment however shows that the ascospores are able to bring about infection on healthy wounded leaves.

VI. CONTROL MEASURES.

Spraying against this disease would not be effective as the parasite is soil-borne. Picking off the lower leaves of tobacco plants may be helpful as this

practice will help in promoting the circulation of fresh air which will reduce the humidity at the surface of the soil, thus preventing the germination of spores and the mycelium. The soil between the plants should be well worked and weeds removed. Infected plants should be collected and burnt in order to prevent the sclerotia from reaching the soil. Plants liable to be attacked by this fungus should not be planted in infested soil for at least three years. Soon after the harvest the plants should be pulled out so as to expose any sclerotia that may lie around the plants and the land kept fallow so that summer heat may inactivate and if possible kill the sclerotia. Experiments described in this paper have shown that they are killed at 30°C. Temperatures above this point do occur regularly in the plains in the hot weather and soil temperature of upper layer of soil may reach up to 50°C. in the course of a day.

VII. Summary.

1. *Sclerotinia sclerotiorum* Lib. (De Bary) causes a stem-rot of tobacco in Rangpur, Bengal.

2. Infection takes place on wounded stems and through the roots in the soil.

3. Infection can be brought about by whole or cut sclerotia on wounded stems as well as by ascospores on wounded leaves.

4. The optimum temperature for the growth of the fungus is between 20° and 25°C. At 30°C. the fungus does not grow.

5. In rich media microconidial development takes place late when the available food is used up by the growing fungus, but in poor media it takes place in a short time.

6. The perfect stage of the fungus with fully matured asci and paraphyses was found.

7. Low temperature is one of the factors in the development of apothecial stalks and light is essential for their expansion into discs.

8. A Botrytis stage was not found in the study of the life history of this fungus.

VIII. Acknowledgments.

The author's thanks are due to Dr. W. McRae, the Imperial Mycologist, for encouraging advice and help in the course of this work. He also acknowledges his indebtedness to Drs. M. Mitra and B. B. Mundkur for help given in this work.

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Explanation of Plates XXXIX—XLII.

PLATE XXXIX.

Fig. 1. Hyphal filaments forming organs of attachment (×720).
Fig. 2. Microconidia developing in 2 per cent potato agar (×720).
Fig. 3. Microconidia developing on mature apothecial discs (×720).
Fig. 4. Sclerotium with fully matured apothecia (×2).
Fig. 5. Small sclerotium showing two apothecial discs (×2).
Fig. 6. Big sclerotium showing many processes but one apothecial disc (×2).

PLATE XL.

Fig. 1. Apothecia growing from sclerotia in culture (×3).
Fig. 2. Apothecia growing from sclerotia on sawdust (×4).

PLATE XLI.

Fig. 1. Ascospores of *Sclerotinia sclerotiorum* Lib. (De Bary) (×720).
Fig. 2. Asci and paraphyses from the apothecial cup of *Sclerotinia sclerotiorum* (×720).
Fig. 3. Sclerotia in the pith of an infected plant (×2).
Fig. 4. Transverse section of an infected stem showing mycelium in the tissues (×720).
Fig. 5. Apothecia growing at the base of an infected plant (×3).

PLATE XLII.

Fig. 1. Artificially infected tobacco plants ($\frac{1}{3}$ actual size).
Fig. 2. Apothecia growing on the surface of an infected soil in a pot ($\frac{1}{2}$ actual size).

INHERITANCE OF CHARACTERS IN *CAJANUS INDICUS*.

BY

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Department of Agriculture, Central Provinces.

(Received for publication on 20th April 1934)

(With Plates XLIII-XLVI)

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I. Introduction.

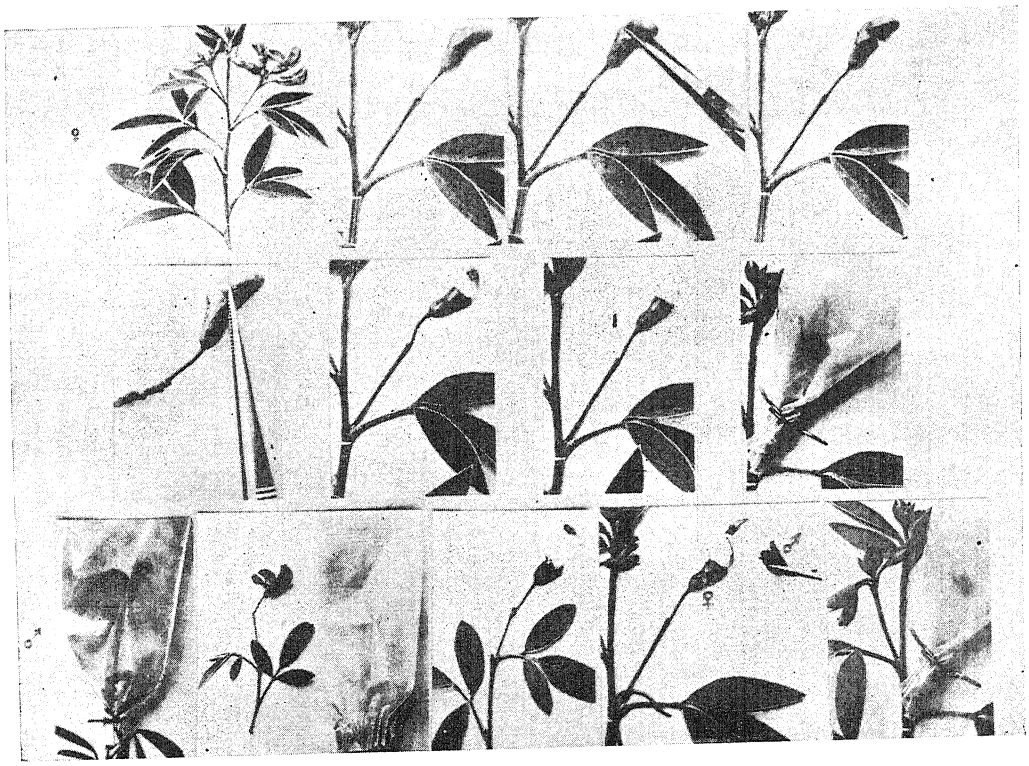
The types of *Cajanus indicus* have been studied in a previous publication [Mahta and Dave, 1931] in which 36 main types of this crop are described. The classification there is based on flower colour, pod colour and seed-coat colour. The present paper furnishes data on the mode of inheritance of these morphological characters.

The method adopted in obtaining the hybrids is clearly illustrated in Plate XLIII.

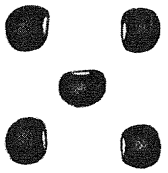
II. Hybridization results and their interpretation.

1. COLOUR OF SEED COAT.

The commonest types of seed-coat colours in *Cajanus indicus* are brown and white in pure and different shades. Sometimes, however, purplish black, dark

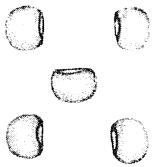


Technique of crossing employed.



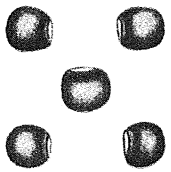
Purplish black ♂

×

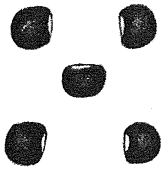


White ♀

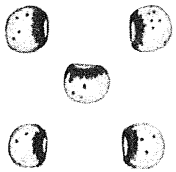
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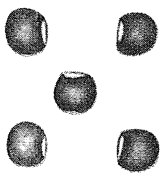
F_2.



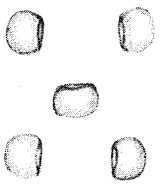
Purplish black.



White with purple spots.



Brown.



White.

mottled brown and distinctly white forms also occur. The last two are very late in ripening and hence have not been used for crossing purposes. The following crosses supply data concerning the inheritance of the colour of seed-coat.

A. White × purplish black.

The F_1 had purplish black seeds and the F_2 segregated into (1) purplish black, (2) white with purple spots, (3) brown and (4) white in proportions suggesting a 9 : 3 : 3 : 1 ratio (Plate XLIV). The details of segregation are given in the following tables :—

	Purplish black	White with purple spots	Brown	White
Frequency observed . .	298	118	102	37
,, expected . .	312·19	104·06	104·06	34·69
Ratio observed . . .	8·59 :	3·40 :	2·94 :	1·07
,, expected . . .	9 :	3 :	3 :	1

$\chi^2 = 2·66$.

The results of F_2 indicate that the inheritance of purplish black colour in the seed coat is determined by the interaction of two factors.

F_3.—Twenty-two families in all could be obtained from selfed seed. Their segregations are given in the following table :—

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
5 cultures from plants with purplish black seeds.	Pure	1
	9 : 3 : 3 : 1 ratio like F_2 . . . . (108 purplish black, 35 white with purple spots, 36 brown and 13 white).	1
	3 purplish black : 1 brown . . . (245 ,, ,, : 83 ,,).	2
	3 purplish black : 1 white with purple spots .	1
7 cultures from plants having white seeds with purple spots.	Pure	3
	3 white with purple spots : 1 white . . (252 ,, ,, ,, ,, : 79 ,,).	4
7 cultures from plants with brown seeds.	Pure	2
	3 brown : 1 white . . . . . (321 ,, : 101 ,,).	5
3 cultures from plants with white seeds.	Pure	3

The results of F_3 support the bifactorial segregation of 9 : 3 : 3 : 1 observed in F_2.

It has not been possible in crosses described here to obtain an adequate number of F_3 families from selfed seed on account of the loss of a number of selfed plants from wilt.

B. Brown × purplish black.

The F_1 showed complete dominance of purplish black colour and the F_2 segregated into purplish black and brown in the following proportions :—

	Purplish black		Brown
Frequency observed	728		239
„ expected	725·25		241·75
Ratio observed	3·01	:	0·99
„ expected	3	:	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{2{\cdot}75}{1{\cdot}346} = 0{\cdot}2$$

The ratio of purplish black to brown is close to the expected 3 : 1, characteristic of a single factor difference.

In F_3 the following segregations occurred :—

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
9 cultures from plants with purplish black seeds.	Pure	3
	3 purplish black : 1 brown	6
	(381 „ „ : 146 „).	
3 cultures from plants with brown seeds.	Pure	3

The existence of a single factor difference between purplish black and brown is hence confirmed.

C. Brown × white.

The F_1 was brown and the F_2 population showed the following frequencies for seed colour :—

	Brown		White
Frequency observed	984		305
„ expected	966·75		322·25
Ratio observed	3·05	:	0·95
„ expected	3	:	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{17{\cdot}25}{10{\cdot}48} = 1{\cdot}64$$

The results obtained suggest a monohybrid type of segregation.

In F_3 the following segregations occurred :—

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
4 cultures from plants with brown seeds.	Pure	2
	3 brown : 1 white . . . . (164 „ : 58 „)	2
2 cultures from plants with white seeds.	Pure	2

The F_3 observations agree with the 3 : 1 ratio of brown to white seeds in F_2.

Interpretation of results.

It is evident from the foregoing data that both purplish black and brown colours of the seed coat are dominant to white. The purplish black is also dominant to brown. The genes for purplish black spotting and brown being designated as **P** and **R** respectively the genetic constitution of the parental types would be :—

RRPP.—Purplish black.

RRpp.—Brown.

rrpp.—White.

In the cross of white × purplish black (**rrpp** × **RRPP**) the factors segregating will be **R** and **P**. The F_1 (**RrPp**) should be purplish black and the F_2 should give : —

Homozygous			Heterozygous			
Genotype	No	Colour	Genotype	No	Colour	Behaviour in F_2
RRPP	1	Purplish black	**RrPP**	2	Purplish black	3 Purple : 1 White with purple spots.
rrPP	1	White with purple spots.	**RRPp**	2	Purplish black	3 Purple : 1 Brown.
RRpp	1	Brown.	**RrPp**	4	Purplish black	9 Purple : 3 Purple spots.
rrpp	1	White.				3 Brown : 1 White.
			rrPp	2	White with purple spots.	3 Purple spots : 1 White.
			Rrpp	2	Brown . .	3 Brown : 1 White.

or a ratio of— $\frac{9\,\mathbf{RP}}{\text{Purplish black}} : \frac{3\,\mathbf{rP}}{\text{White with purple spots}} : \frac{3\,\mathbf{Rp}}{\text{Brown}} : \frac{1\,\mathbf{rp}}{\text{White}}$ which agrees with the results obtained.

In the cross of brown × purplish black (**RRpp** × **RRPP**) the F_1 is heterozygous only for **P** and would be purplish black. The segregation of this factor will give in F_2 a ratio of $\frac{1\ \mathbf{PP}:\ 2\ \mathbf{Pp}}{3\ \text{Purple}} : \frac{1\ \mathbf{pp}}{1\ \text{Brown}}$ which is fully realised.

In the cross of brown and white (**RRpp** × **rrpp**) the factor segregating is **R**. The F_1 should be brown and in F_2 a ratio of 3 brown : 1 white should be obtained. This agrees with the results obtained.

2. Pod colour.

The colours of the pod in *Cajanus indicus* are of three kinds. (1) Green with purplish black streaks to which the name of green blotched with maroon is given, (2) green and (3) a diffused purplish black colour all over the pod described in this paper as dark. These refer to the colours of the pods in the unripe stage which on drying lose their greenness but retain their maroon colouring.

The following crosses supply data concerning the inheritance of the colour of pod.

A. Green × dark.

The F_1 had darkish pods lighter in colour than the dark parent. In F_2 segregation occurred into dark, maroon-blotched and green in the ratio of 9 : 3 : 4. (Plate XLV).

	Dark	Maroon-blotched	Green
Frequency observed	647	232	286
„ expected	655·31	218·44	281·25
Ratio observed	8·88 :	3·19 :	3·93
„ expected	9 :	3 :	4

$\chi^2 = 1{\cdot}04$

The results obtained indicate a dihybrid type of segregation in pod colour.

In F_3 the following segregations were observed.

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
5 cultures from plants with dark pods.	Pure	2
	9 Dark : 3 Maroon-blotched : 4 Green (138 „ : 48 „ „ : 56 „)	2
	3 Dark : 1 Green	1
4 cultures from plants with maroon-blotched pods.	Pure	2
	3 Maroon-blotched : 1 Green . . (285 „ „ : 81 „	2
4 cultures from plants with green pods.	Pure	4

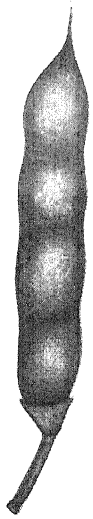


Dark ♂

×



F_1.



Green ♀



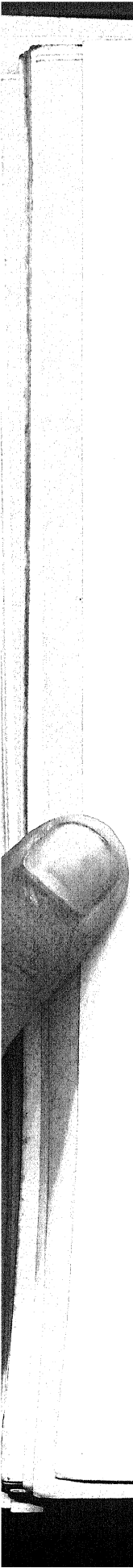
Dark.



Homozygous forms in F_2.
Blotched with maroon.



Green.



The figures of F_3 support the bifactorial segregation of 9 : 3 : 4 observed in F_2.

B. Green × maroon-blotched.

The F_1 had maroon-blotched pods and the F_2 segregated into maroon-blotched and green in the ratio of 3 : 1.

	Maroon-blotched		Green
Frequency observed	1275		446
„ expected	1290·75		430·25
Ratio observed	2·97	:	1·03
„ expected	3	:	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{15{\cdot}75}{12{\cdot}11} = 1{\cdot}30$$

From the above figures it is evident that maroon-blotched and green behave as simple allelomorphs.

In F_3 twelve families were raised. The details of segregation are given below—

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
9 cultures from plants with maroon-blotched pods.	Pure	3
	3 Maroon-blotched : 1 Green	6
	(463 „ „ : 149 „)	
3 cultures from plants having green pods.	Pure	3

The existence of a single factor difference between maroon-blotched and green is hence confirmed.

C. Dark × maroon-blotched.

The F_1 gave dark pods and the F_2 segregated into dark and maroon-blotched in the ratio of 3 : 1.

	Dark		Maroon-blotched
Frequency observed	1431		485
„ expected	1437		479
Ratio observed	2·99	:	1·01
„ expected	3	:	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{6}{12{\cdot}78} = 0{\cdot}46.$$

In seventeen cultures in F_3 the following segregations occurred.

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
13 cultures from plants having dark pods.	Pure	5
	3 Dark : 1 Maroon-blotched . . .	8
	(369 „ : 112 „ „) . . .	
4 cultures from plants with maroon-blotched pods.	Pure	4

The results of F_3 confirm the 3 : 1 ratio of dark to maroon-blotched observed in F_2.

Interpretation of results.

The inheritance of pod colour in *Cajanus indicus* depends upon the interaction of two factors—

L—a factor which causes maroon blotching.

D—a factor which extends the maroon blotching all over the pod. This factor has no visible effect except in presence of **L**.

According to this hypothesis the factorial constitution of the various types would be :—

LLDD—Dark.

LLdd—Maroon-blotched.

lldd—Green.

In the cross green×dark (**lldd**×**LLDD**) the factors segregating are **L** and **D**. The F_1 (**LlDd**) should have dark pods and the F_2 should show a dihybrid segregation of

$$\frac{9\ \mathbf{LD}}{9\ \text{Dark}} : \frac{3\ \mathbf{Ld}}{3\ \text{Maroon blotched}} : \frac{3\ \mathbf{lD} : 1\ \mathbf{ld}}{4\ \text{Green}}$$

This is confirmed by the results obtained.

In the cross green×maroon-blotched (**lldd**×**LLdd**), there is a single factor segregation of **L**. The F_1 (**Lldd**) should have maroon-blotched pods and in F_2, maroon-blotched and green should appear in the ratio of 3 : 1. The observed frequencies agree with this.

In the cross dark×maroon-blotched (**LLDD**×**LLdd**) the parents are homozygous for the factor **L**. The segregation of **D** alone should give in F_1 (**LLDd**) dark pods

and in F_2 dark and maroon-blotched in the ratio of 3 : 1. The results obtained confirm this.

3. Flower colour.

The standard of the papilionaceous flower in this crop generally is either plain yellow or yellow veined with red. In addition there are three other forms often met with, *viz.*, orange, purple and yellow having purple veins with base diffused purple. Crosses have been made amongst the various types described above with the object of studying their mode of inheritance and the results of each are recorded below.

A. Flowers yellow, back of standard with self coloured veins. × *orange yellow.*

The flower colour of the first hybrid generation was quite indistinguishable from that of the orange flowered parent. Orange is thus completely dominant over the yellow. In F_2 segregation occurred into the parental types in the following proportions :—

	Orange		Yellow
Frequency observed	430		146
„ expected	432		144
Ratio observed	2·99	:	1·01
„ expected	3	:	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{2{\cdot}00}{7{\cdot}01} = 0{\cdot}28.$$

The ratio of orange to yellow is close to the simple Mendelian 3 : 1 ratio characteristic of a single factor difference.

In F_3 the following segregations occurred :—

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
3 cultures from plants with orange flowers.	Pure	1
	3 Orange : 1 Yellow	2
	(365 „ : 123 „).	
2 cultures from plants with yellow flowers.	Pure	2

The results of F_3 confirm the 3 : 1 ratio of orange to yellow observed in F_2.

B. Yellow, back of standard with self coloured veins × yellow, back of standard purple.

The F_1 had light purple standard with deep purple veins. In F_2 the ratio of purple and veined with purple to yellow was 3 : 1. The details of segregation are given below :—

	Purple and veined with purple.		Yellow.
Frequency observed	186		46
„ expected	174		58
Ratio observed	3·21	:	0·79
„ expected	3	:	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{12}{4{\cdot}44} = 2{\cdot}69$$

In F_3 the following segregations occurred :—

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
2 cultures from plants with purple standard.	Pure	2
2 cultures from plants having light purple standard with deep purple veins.	3 Purple : 1 Yellow (163 „ : 63 „).	2
2 cultures from plants with yellow standard.	Pure	2

The F_3 observations agree with the 3 : 1 ratio of purple to yellow obtained in F_2.

In a second cross of 'yellow' × 'purple' the F_1 had light purple standard with deep purple veins but the F_2 segregated into purple and yellow in the ratio of 9 : 7 :—

	Purple and veined with purple		Yellow
Frequency observed	244		195
„ expected	246·94		192·06
Ratio observed	8·89	:	7·11
„ expected	9	:	7

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{2{\cdot}94}{6{\cdot}87} = 0{\cdot}427$$

The F_3 segregations are given in the following table :—

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
3 cultures from plants with purple standard.	3 Purple : 1 Yellow . . . . (219 „ : 76 „).	3
3 cultures from plants having light purple standard with deep purple veins.	9 Purple : 7 Yellow . . . . (140 „ : 125 „).	3
3 cultures from plants with yellow standard.	Pure	3

The F_3 observations agree with the 9 : 7 ratio of purple to yellow observed in F_2.

C. Yellow, back of standard with red veins × *orange yellow.*

The F_1 had orange yellow flowers and the F_2 segregated into orange and yellow veined with red in the ratio of 3 : 1.

	Orange		Yellow veined with red
Frequency observed	479		177
„ expected	492		164
Ratio observed	2·92	:	1·08
„ expected	3	:	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{13}{7{\cdot}48} = 1{\cdot}73$$

The F_3 results are as follows : —

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
4 cultures from plants with orange flowers.	Pure	2
	3 Orange : 1 Yellow veined with red .	2
	(298 „ : 116 „ „ „ „) .	
3 cultures from plants having yellow flowers with red veins.	Pure	3

The F_3 observations agree with the 3 : 1 ratio of orange to yellow veined with red obtained in F_2.

D. Yellow, back of standard with purple veins, base diffused purple × yellow, back of standard purple.

The F_1 had light purple standard with deep purple veins. In F_2 the ratio of purple to yellow veined with purple, base diffused purple was 3 : 1.

	Purple and light purple, veins deeper		Yellow with purple veins, base diffused purple
Frequency observed	152		56
„ expected	156		52
Ratio observed	2·92	:	1·08
„ expected	3	:	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{4{\cdot}00}{4{\cdot}21} = 0{\cdot}949$$

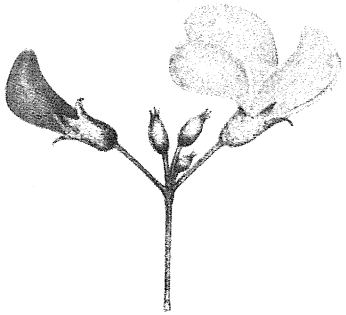
In F_3 the following segregations were observed :—

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
2 cultures from plants with purple standard.	Pure	2
2 cultures from plants with light purple standard.	3 Purple : 1 Yellow veined with purple, base diffused purple.	2
	(167 Purple : 55 Yellow veined with purple base diffused purple.)	
2 cultures from plants with yellow standard having purple veins, base diffused purple.	Pure	2

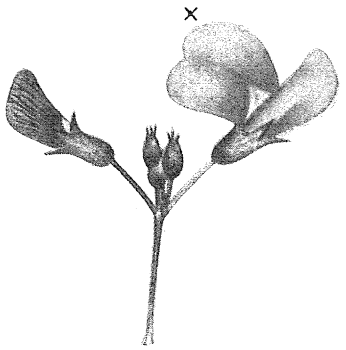
The results of F_3 support the 3 : 1 ratio of purple to yellow veined with purple, base diffused purple observed in F_2.

E. Orange yellow × yellow, back of standard purple.

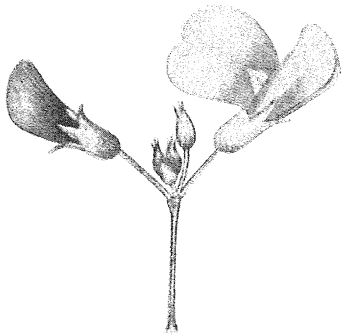
F_1.—The colour on the dorsal surface of standard was inherited independently of that on the ventral side. On the dorsal side purple was dominant to orange and on the ventral, orange was dominant to yellow. The F_1 had flowers with back of standard purple and front orange (Plate XLVI).



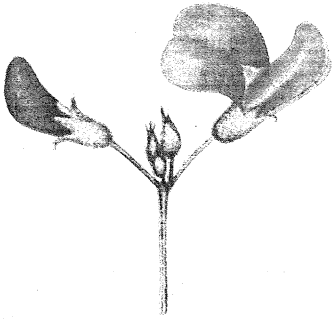
Flowers yellow, dorsal surface of standard purple ♂



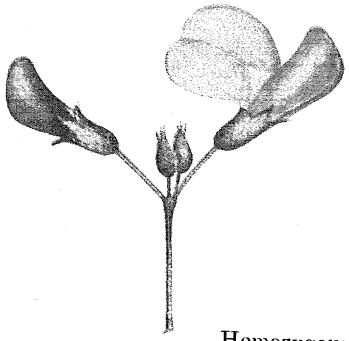
F_1.
Dorsal surface of standard purple, with deeper veins. Ventral surface orange.



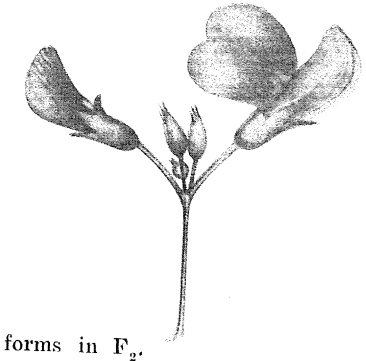
Orange yellow ♀



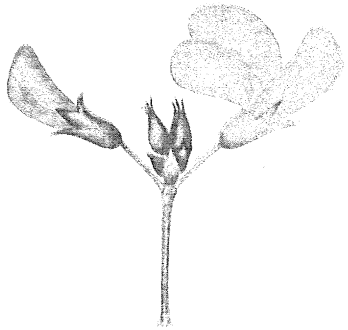
D. S. = Purple.
V. S. = Orange.



D. S. = Purple.
V. S. = Yellow.



Orange.



Yellow.

Homozygous forms in F_2.

PLATE XLVI.

F_2.—On the dorsal side, the segregation of purple gave in F_2 a ratio of 12 Purple and veined with purple, base diffused purple : 3 Orange : 1 Yellow. On the ventral side the segregation of orange gave a ratio of 3 Orange : 1 Yellow. The details of these are given below :—

Dorsal side . . .	Purple and veined with purple	Purple and veined with purple	Orange	Yellow
Ventral side . . .	Orange	Yellow	Orange	Yellow
	289	78	89	27

Dorsal side—

	Purple and veined with purple	Orange	Yellow
Frequency observed	367	89	27
„ expected	362·25	90·56	30·19
Ratio observed	12·16 :	2·95 :	0·89
„ expected	12 :	3 :	1

$\chi^2 = 0{\cdot}425$.

Ventral side—

	Orange	Yellow
Frequency observed	378	107
„ expected	363·75	121·25
Ratio observed	3·12 :	0·88
„ expected	3 :	1

$$\frac{\text{Deviation}}{\text{Probable error}} = \frac{14{\cdot}75}{6{\cdot}42} = 2{\cdot}295$$

F_3 —In twenty-six cultures in F_3 the following segregations occurred.

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
5 cultures from plants with back of standard purple and front orange.	Pure	3
	Dorsal side— Pure—	2
	Ventral side—3 Orange : 1 Yellow . .	
	(151 „ : 54 „)	
3 cultures from plants with back of standard veined with purple, base diffused purple ; front orange.	Dorsal side—12 Purple : 3 Orange : 1 Yellow.	3
	(97 „ : 21 „ : 6 „)	
	Ventral side—3 Orange : 1 Yellow.	
	(93 „ : 21 „)	

Number of cultures and nature of parent plant in F_2	Segregations	Frequencies
4 cultures from plants with back of standard purple, front yellow.	Pure	4
1 back of standard veined with purple, base diffused purple, front yellow.	Dorsal side—3 Purple : 1 Yellow. (55 „ : 22 „) Ventral side pure.	1
8 cultures from plants with yellow flowers.	Pure	8
5 cultures from plants with orange flowers.	Pure	2
	3 Orange : 1 Yellow.	3
	(151 „ : 47 „)	

The results of F_3 support the 12 : 3 : 1 ratio of purple, orange and yellow on the dorsal surface of standard and the 3 : 1 ratio of orange and yellow on the ventral surface as observed in F_2.

Interpretation of results.

A study of the above crosses has revealed the existence of numerous factors controlling the colour of standard in the flower of *Cajanus indicus*. It has been found that purple colour at the back of standard is dominant to plain yellow, to yellow with purple veins, base diffused purple and also to orange yellow, and that orange is dominant to plain yellow and to yellow with red veins. On the basis of segregations observed in F_2 and F_3 the existence of the following factors may be suggested ;—

P—a factor for orange colour. In presence of **A** it is intensified into deep orange.

A—a basic factor necessary for the development of red or purple.

C—a factor which acts with **A** to produce purple colour in the veins and diffused purple at the base of standard.

E—a factor which in presence of **A** and **C** extends purple colour all over the standard.

V—a factor which acts with **A** to produce red colour in the veins.

According to this hypothesis the genetic constitution of the various types of standard colours will be as follows :—

AAPPcceevv—Orange yellow.

AACCEEppvv—Purple.

AACCeeppvv—Yellow with purple veins, base diffused purple.

AAVVcceepp—Yellow with red veins.

AAcceeppvv
aaccceeppvv } —Yellow.

The genetic relation existing between the various types may now be considered.

Yellow, back of standard with self coloured veins × *orange yellow.*

In this cross (**AAcceeppvv** × **AAPPcceevv**) the F_1 is heterozygous only for the factor **P** and should be orange. The segregation of this factor should give in F_2 a ratio of 3 orange to 1 yellow which is fully realised.

Yellow, back of standard with self coloured veins × *yellow, back of standard purple.*

In the cross **AAcceeppvv** × **AACCEEppvv** the F_1 is heterozygous for the factors **C** and **E** and should be light purple with deeper veins. The segregation of these factors should give in F_2 a ratio of :—

$$\frac{9\ \mathbf{CE}}{9\ \text{Purple}} : \frac{3\ \mathbf{Ce}}{3\ \text{Purple veins, base diffused purple}} : \frac{3\ \mathbf{cE} : 1\ \mathbf{ce}}{4\ \text{Yellow}}$$

or 12 purple and veined with purple, base diffused purple : 4 yellow which is realised.

In the other cross of yellow × purple (**aaccceeppvv** × **AACCEEppvv**) the F_1 is heterozygous for **A, C,** and **E** and should be light purple with deeper veins. The segregation of these three factors should give in F_2 a ratio of :—

$$\frac{27\ \mathbf{ACE}}{27\ \text{Purple}} : \frac{9\ \mathbf{ACe}}{9\ \text{Purple veins, base diffused purple}} : \frac{9\ \mathbf{AcE}}{9\ \text{Yellow}} :$$

$$\frac{9\ \mathbf{aCE}}{9\ \text{Yellow}} : \frac{3\ \mathbf{Ace}}{3\ \text{Yellow}} : \frac{3\ \mathbf{aCe}}{3\ \text{Yellow}} : \frac{3\ \mathbf{acE}}{3\ \text{Yellow}} : \frac{1\ \mathbf{ace}}{1\ \text{Yellow}}$$

or 36 purple and veined with purple base diffused purple : 28 yellow *i.e.* a ratio of 9 purple to 7 yellow which agrees with the results obtained.

Yellow, back of standard red veined × *orange yellow.*

In the cross **AAVVcceepp** × **AAPPcceevv,** the F_1 is heterozygous for the factors **P** and **V** and should be orange. The segregation of these two factors should give in F_2 a ratio of :—

$$\frac{9\ \mathbf{PV} : 3\ \mathbf{Pv}}{12\ \text{Orange}} : \frac{3\ \mathbf{pV} : 1\ \mathbf{pv}}{4\ \text{Red veined and yellow}}$$

which agrees with the results obtained.

Yellow, back of standard with purple veins, base diffused purple × yellow, back of standard purple.

In this cross the F_1 (**AACCEeppvv**) is homozygous for all the factors except **E** and should have light purple standard with deep purple veins. The F_2 should give a ratio of 3 Purple : 1 Veined with purple, base diffused purple, which is realised.

Orange yellow × yellow, back of standard purple.

In the cross **AAPPeeeevv × AACCEEppvv**, the F_1 is heterozygous for the factors **C**, **E**, and **P** and should have dorsal surface of standard purple and ventral surface orange. The segregation of these factors in F_2 should give :—

$$\frac{27\ \textbf{CEP}}{\text{Purple}} : \frac{9\ \textbf{CEp}}{\text{Purple}} : \frac{9\ \textbf{CeP}}{\text{Purple veins, base diffused purple}} : \frac{9\ \textbf{cEP}}{\text{Orange}} :$$

$$\frac{3\ \textbf{Cep}}{\text{Purple veins, base diffused purple}} : \frac{3\ \textbf{cEp}}{\text{Yellow}} : \frac{3\ \textbf{ceP}}{\text{Orange}} : \frac{1\ \textbf{cep}}{\text{Yellow}}$$

or a ratio of 12 Purple : 3 Orange : 1 Yellow on the dorsal side of standard and a ratio of 3 Orange : 1 Yellow on the ventral surface. This is in accordance with the results obtained.

III. Linkage of characters in inheritance.

Some evidence of linkage between flower colour and pod colour and flower colour and seed-coat colour has been obtained during the study of inheritance in *Cajanus indicus*. It was found that :—

1. Orange yellow flowers and purplish black seeds were completely linked in inheritance.
2. The factors for purple colour at the back of standard were closely linked with the one for maroon colour of the pod.
3. Complete linkage was present between yellow flowers with back of standard having purple veins, base diffused purple and green pods.

1. Linkage of orange yellow flowers and purplish black seeds.

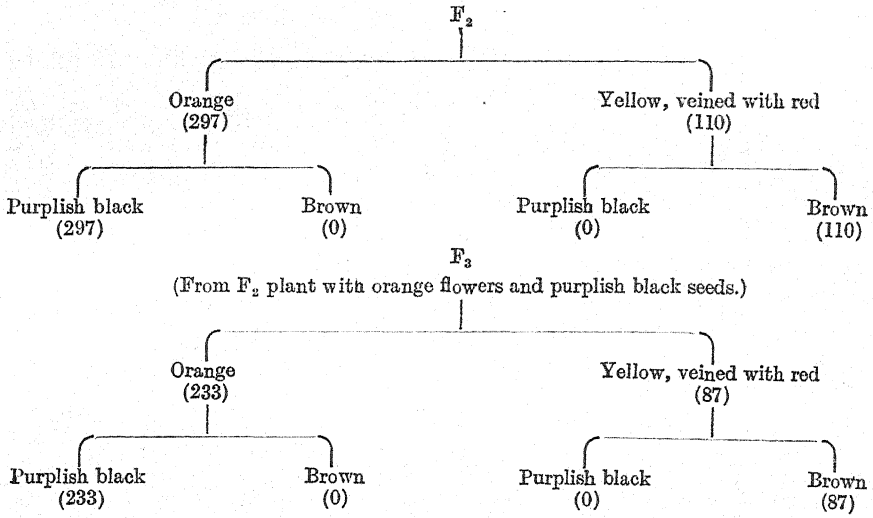
In the cross of Yellow, back of standard with red veins × Orange it has been shown that orange colour of the flower depends for its expression on a single factor, so that in F_2 segregation is in the ratio of 3 Orange : 1 Yellow For purplish black seeds, when contrasted with brown, it has been shown that purplish black is dominant and that in F_2 segregation is in accordance with the normal monohybrid ratio—3 Purplish black : 1 Brown. When a type having yellow flowers with back of standard veined with red and brown seeds is crossed with another having orange flowers and purplish black seeds, the F_1 has orange flowers and purplish black seeds but the F_2 does not show the expected ratio of 9 Orange purple : 3

Orange brown : 3 Yellow purple : 1 Yellow brown. The data actually obtained are given below.

Flowers yellow, back of standard veined with red, seeds brown.

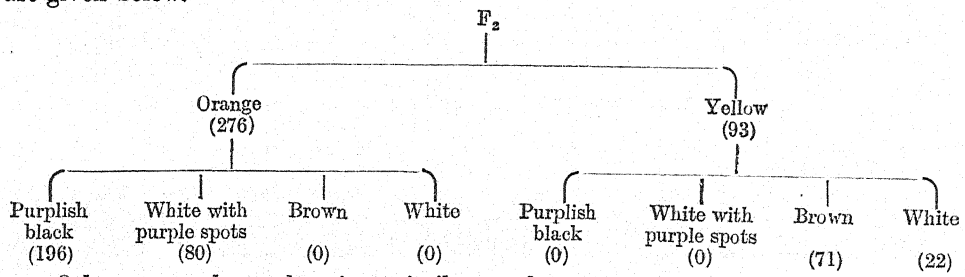
×

Flowers orange, seeds purplish black.



Taking each pair of characters separately, the agreement with the monohybrid ratio is very satisfactory but the absence of the phenotypes--orange flower, brown seed and yellow flower, purplish black seed indicates that orange flowers and purplish black seeds are completely linked in inheritance.

A second cross of a yellow flowered white seeded type with an orange flowered purplish black seeded type gave similar results. The F_2 segregations of this cross are given below.



Other crosses have also given similar results. Orange flowers and purplish black seeds are therefore completely linked in inheritance.

2. Linkage of purple standard and maroon-blotched pod.

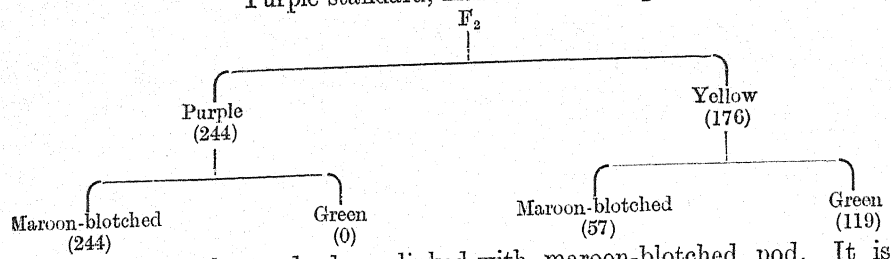
In a cross between a type having yellow standard and green pod and a type with purple standard and maroon-blotched pod, the F_2 segregations showed a 9 : 7

ratio for purple standard *versus* yellow and a 3 : 1 ratio for maroon-blotched pod *versus* green. The segregates with purple standard always had maroon-blotched pods. The F_2 ratios were as follows :—

Yellow standard, green pod.

×

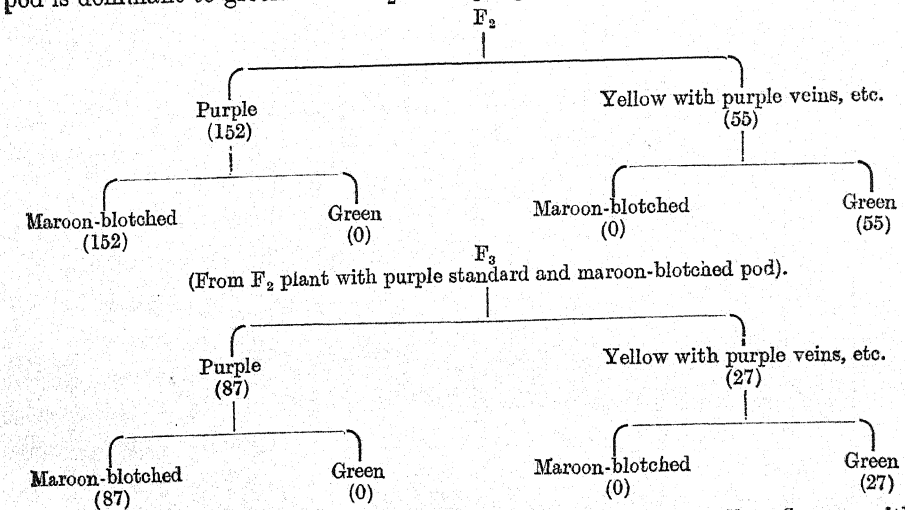
Purple standard, maroon-blotched pod.



In F_3 also purple standard was linked with maroon-blotched pod. It is thus fairly clear that these two characters are very closely linked in inheritance.

3. Linkage between yellow flowers with back of standard having purple veins, base diffused purple and green pods.

This type of linked inheritance is illustrated by the cross yellow flower, back of standard with purple veins, base diffused purple and green pod×yellow flower with back of standard purple and maroon-blotched pod. Purple standard is a simple dominant to yellow with purple veins, base diffused purple and maroon-blotched pod is dominant to green. The F_2 and F_3 segregations of this cross are given below.



F_3

(From F_2 plant with purple standard and maroon-blotched pod).

The results given above show a complete linkage between yellow flowers with back of standard having purple veins, base diffused purple and green pods. Other three crosses have also shown complete linkage between the two characters mentioned above,

Summary.

The inheritance of flower colour, pod colour and seed-coat colour in *Cajanus indicus* has been described. The types of standard colour studied are purple, orange, plain yellow, yellow with red veins and yellow with purple veins, base diffused purple. Crosses of yellow and orange flowers gave orange in F_1 and a 3 : 1 ratio of orange and yellow in F_2. The F_3 segregations were in accordance with the expectations. Crosses of plain yellow and yellow with back of standard purple gave light purple with deeper veins in F_1 and a 3 : 1 ratio of purple to yellow in F_2. In certain crosses a 9 : 7 ratio of purple and yellow was also obtained. Crosses of yellow with back of standard red veined and orange gave in F_1 orange and in F_2 a simple segregation of orange and red veined. Crosses of purple and yellow with purple veins, base diffused purple gave light purple with deeper veins in F_1 and a 3 : 1 ratio in F_2. Crosses of yellow, back of standard purple and orange gave in F_1 flowers with dorsal surface of standard purple and ventral surface orange. In F_2 the ratio of these colours on the dorsal surface of standard was 12 Purple : 3 Orange : 1 Yellow. On the ventral side, the segregation of orange gave orange and yellow in the ratio of 3 : 1.

The types of pod colour studied are dark, maroon-blotched and green. Crosses of green × dark gave dark in F_1 and a 9 : 3 : 4 ratio of dark, maroon-blotched and green in F_2. In crosses of dark × maroon-blotched and maroon-blotched × green, the segregations obtained indicated a monohybrid inheritance.

The types of seed-coat colour studied are purplish black, brown and white. Crosses of brown and white gave brown in F_1 and a ratio of 3 Brown to 1 White in F_2. In crosses of purplish black and white the F_1 was purplish black and gave in F_2 a ratio of 9 Purplish black : 3 White with purple spots : 3 Brown : 1 White. Crosses of brown and purplish black gave purplish black in F_1 and a simple 3 : 1 ratio of Purplish black and Brown in F_2.

Orange yellow flowers and purplish black seeds were completely linked in inheritance. Purple colour at the back of standard was closely linked with maroon colour of the pod. Complete linkage was also present between yellow flowers with back of standard having purple veins, base diffused purple and green pods.

The work described in this article was carried out under the direction of Mr. D. N. Mahta, Economic Botanist to Government, C. P., and I am much indebted to him for valuable suggestions in the interpretation of genetic results embodied in this paper.

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A LEAF SPOT DISEASE OF WHEAT CAUSED BY *HELMINTHOSPORIUM TRITICI-REPENTIS* DIED.

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(With two text-figures)

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I. INTRODUCTION

A large number of species of *Helminthosporium* have been recorded on wheat from various parts of the world, such as *H. tritici* P. Henn. [Hennings, 1903], *H. sativum* P.K. & B., *H. tritici-vulgaris* Nisikado [Nisikado, 1929,1], *H. tetramera* McKinney [McKinney, 1925], *H. pedicellatum* Henry [Henry, 1924] and a few others not fully named. *H. gramineum* Rabh. has also been recorded on wheat by Johnson [1914], Palm [1918] and others, but the description and the illustrations show that these authors were dealing with *H. sativum* and not with *H. gramineum*. In addition to the above there are some species described on wheat which on morphological grounds, such as the detailed structure of the conidiophores and conidia, are more correctly placed in the genus Acrothecium, *e.g.*, *Helminthosporium* M. by Henry [1924] and *H. geniculatum* Tracy and Earle by Palm [1918]. The distinguishing characters of this genus have been cleared up by Mason [1928].

In India *H. sativum* has been recorded on wheat from various provinces including Burma, and recently two new species have been described by the writer [Mitra, 1931] associated with the 'foot-rot' and 'root-rot' diseases of wheat, *viz.*, *H. bicolor* Mitra and *H. halodes* Drechs. var. *tritici* Mitra. The latter resembles very much *H. halodes* described by Drechsler [1923] on *Distichlis spicata*. A fourth species has also been recorded forming spots on leaves and leaf-sheaths and occurs every year in Pusa and in some years does a good deal of damage [Mitra, 1931,2].

This species resembles *H. sativum* in external symptoms but differs widely in morphology. It also differs from all the species recorded on wheat except *H. tritici-vulgaris* described from Japan by Nisikado [1929,2] to which it is closely allied but agrees more closely with *H. tritici-repentis* known to occur on *Agropyron repens* in morphology.

II. Symptoms.

The disease usually appears in the early stages of the growth of wheat seedlings, forming small (1-2 mm.) yellowish. oval to oblong spots on leaves and leaf-sheaths. The spots gradually increase in size and when mature are light brown to dark brown, oval or fusiform and may become irregular, due to the coalescence of several spots. The central region is straw to greyish brown in colour and bears conidiophores and conidia. The margin of the spot is in the form of a yellowish zone which gradually merges into the normal green of healthy leaves. Spots formed by this fungus are very similar to those formed by *H. sativum*, on the leaves of this host and sometimes the two species could only be distinguished from each other on microscopic examination.

III. Economic importance.

Helminthosporium sativum and *Helminthosporium tritici-repentis* do a good deal of damage to wheat in Pusa. The spots formed by these two species of *Helminthosporium* are so similar that no separate estimate of the percentage of leaf area destroyed could be made. In some years one species is far more common than the other, *e.g.*, during 1928-29 and 1930-31 *H. tritici-repentis* was present to a much greater extent than *H. sativum*. During 1928-29 they were present in an epidemic form on wheat in Pusa and its vicinity. Measurements were made of the leaf area covered by the spots caused by these two species by comparing large numbers of leaves picked at random with type leaves whose infected area had been found by measurement according to Tehon [1926] and the percentage of leaf area destroyed was 40 per cent. in Pusa 4, 33 per cent. in Pusa 111, 31 per cent. in Federation bearded, 18 per cent. in Pusa 52 and 16 per cent. in Pusa 12. The disease was again bad during 1929-30 but slightly less than in the previous year, Pusa 4 having 37·5 per cent. and the percentage of leaf area destroyed in other varieties ranging from 3·3 to 17·9. It was much less during 1930-31, 1931-32 and 1932-33. The disease is always less on local country wheat in which the highest percentage of leaf area destroyed was 3·2.

IV. Morphology of the parasite on the host.

Conidiophores.—Conidiophores are formed on the central dead straw coloured portion of the spot and emerge generally from the stomata or sometimes from between epidermal cells either singly or in groups of two to three. They are un-

branched, olive to dark olivacious, 3-9 septate, 38·5-300μ in length and 6-11μ in diameter. The basal cell of the conidiophore is distinctly swollen and is 11-17μ in diameter. The geniculation associated with the production of successive conidia is not generally very pronounced.

Conidia.—Conidia are borne singly at the tip of the conidiophore and become lateral as the conidiophore grows. They are typically subhyaline, straight and cylindrical, 2-11 septate, with an average 6, mode at 5 and septa slightly constricted. The proximal portion of the basal segment of the conidia tapers abruptly in the manner of a cone to be rounded off suggesting somewhat the appearance of the head of a snake, while the distal end of the spore is hemispherical. Conidia show a large variation in size, in length 45-201·5μ with an average of 117·17μ and mode at 125, in width 13-22μ with an average of 16·6 and mode at 16·7. Fig. 1 illustrates the spore, shape and septation.

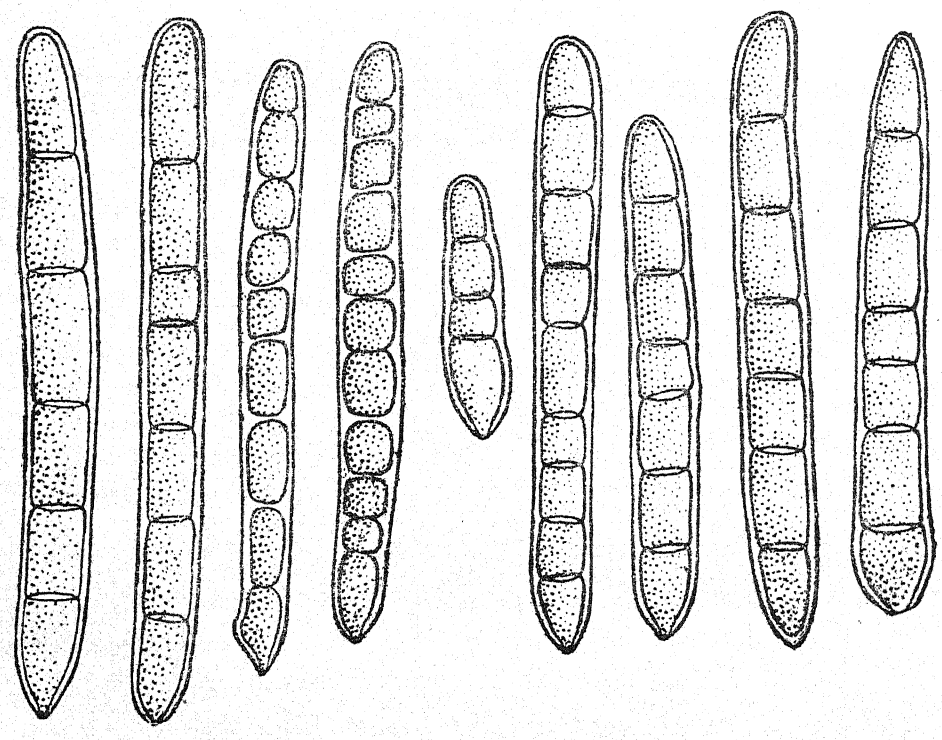


Fig. 1.—Conidia showing shape and septation (×750).

The following table gives the measurements of conidia from the host, and is based on a measurement of three hundred conidia.

Length, width and number of septation of the conidia.

Range	Mode	Mean	Standard deviation	Variation coefficient
Length n iμ 45-201 . . .	125	117·17±1·320	33·9±·93	0·28
Width in μ 13·2-22 . . .	16·7	16·59± ·062	1·60±·044	9·6
Septation 2-11	5	6·00± ·059	1·51±·042	25·1

Spore germination.—The germination of spores takes place within an hour of their being placed in a drop of water, and each segment is capable of germinating. Very often all the cells of a spore have been seen to give out germ tubes and sometimes two or more germ tubes come out from one cell. As in the case of *Helminthosporium bromi* Died. [Drechsler, 1923] so also here, anastomosing of newly proliferated germ tube is not uncommon. When spores are mounted in water and in case they come to lie side by side in contact with each other, pairs of germ tubes proliferate from opposite positions and immediately anastomose, thus uniting the two spores by several short hyphal connections giving the appearance of a scalariform figure. This anastomosing also occurs between germ tubes of two spores lying apart. Fig. 2 illustrates the germination and formation of spores.

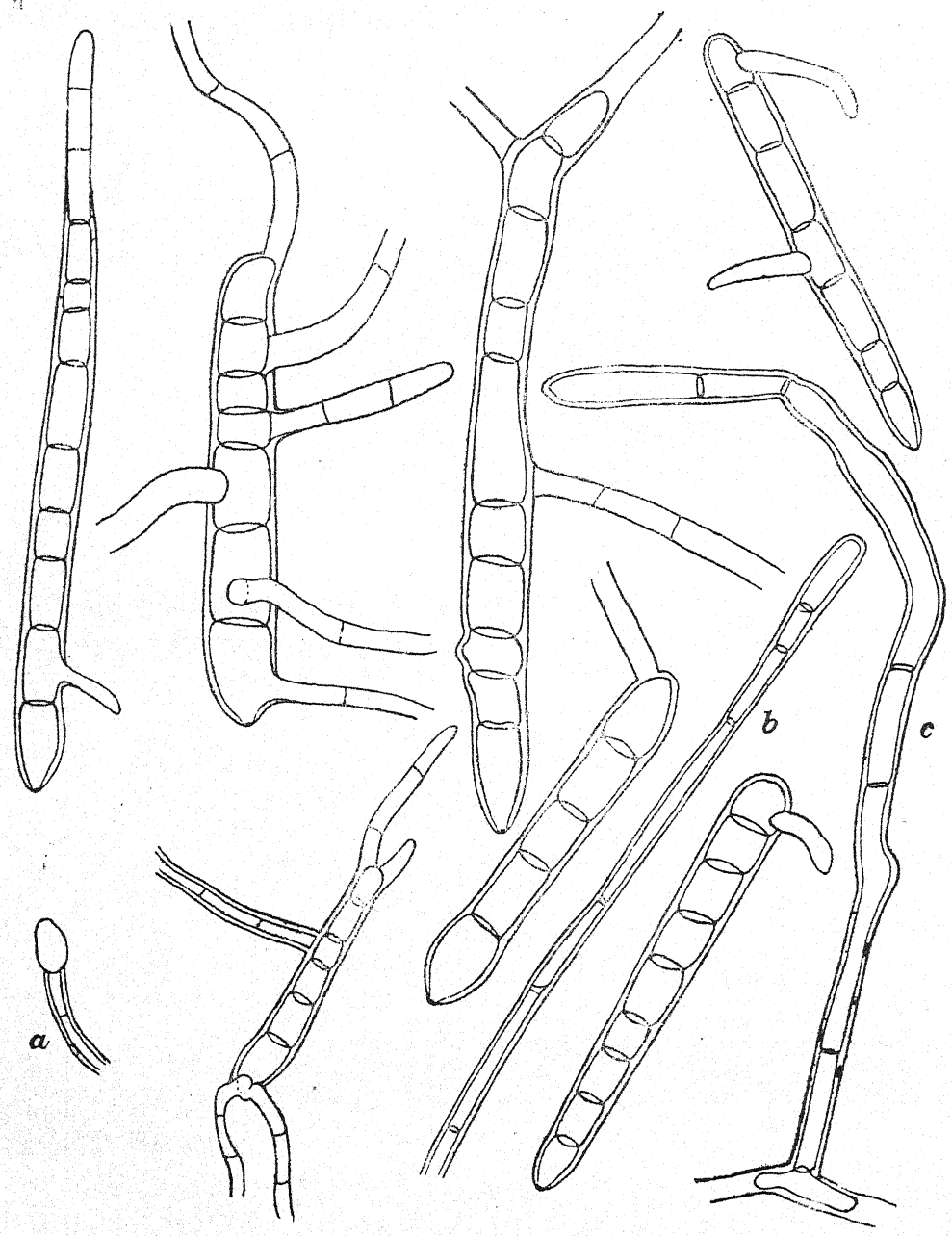


Fig. 2.—Conidia showing shape, septation and germination. *a*, *b*, and *c* show formation of conidiophores and abnormal conidia in culture (×656).

Secondary spores.—Proliferation of spores is a very common thing and has been noticed in a large number of spores of many species of *Helminthosporium*. In this species it is very common and gives rise to spores in chain in the manner of *Alternaria*. The secondary spores are generally smaller in size than the primary ones and are produced directly at the tip. They are also seen to be formed on germ tubes given out by one of the middle segments. The process is repeated several times and thus a large number are produced in chain, each succeeding one smaller in size and less septate. Spores are thin-walled and so short-lived that in a few weeks old specimens some of the segments begin to die and in two-month old specimens the germination percentage of spores is reduced, and it is rather difficult to get germination from six-month old spores.

V. Culture.

A single spore culture of the fungus was obtained several times, and the purity of the culture was guaranteed by taking hyphal tips of growing young mycelium according to the method described by Brown [1924]. In all cases they were identical. The fungus was cultivated on a number of media such as Brown's agar, synthetic starch agar, Richards' solution agar, oatmeal agar, plain agar, etc. On all these media a profuse white cottony aerial growth, somewhat compact, is formed. On plain agar the cottony growth is less and more spreading. The submerged mycelium shows abundant anastomosis with the formation of numerous dark coloured, inflated or lobulate segments. This anastomosis with the resultant production of groups of inflated, lobulate segments is not only abundant in the submerged mycelium of various agar media but also occurs if mycelium is placed on a slide in a liquid culture. The mycelial cells are seen to anastomose, swell up and have a tendency to form mass or pseudo-parenchyma. These apparently represent incipient stages of sclerotia which, as will be mentioned later on, are visible to the naked eye on straw cultures.

Though the fungus grew very well on all the agar media tried, in none of them conidial formation was noticed. Sometimes abnormal spore-like structures with a few septa were seen but they were generally of the same size, thickness and colour as the cells of mycelium, and so it was difficult to say whether they are modified hyphal cells or abnormal spores (Fig. 2). Though normal spores have not been noticed in culture media, but if a little of mycelium is placed on fresh green wheat leaves, spores are formed in large numbers in a day or two. Also, if mycelium with little agar from a culture in which no spores are formed is incubated in a moist chamber, normal spores are readily formed. The fungus was also cultivated on sterilized straw of barley, oats and couch grass. On these the fungus formed a white cottony growth. The fungus penetrates the straw and leaves and on the surface forms

conidiophores and conidia. This formation of conidia is more if fresh straw is sterilized and fungus grown on it. In addition to this spore formation which is of normal size and septation, a very large number of dark coloured sclerotial or immature perithecia are formed either on the straw or embedded in the leaf tissue. These have setae on the surface and also bear conidiophores and conidia in large clusters on the upper surface, which perhaps is the cause of cessation of its further development and checks the formation of ascus and ascospores. The sclerotial-like bodies are hard and brittle and, when crushed, show pseudo-parenchymatous structure. These are also formed on plants inoculated when the tissue has somewhat decayed. They in rare cases only have been seen to produce ascus and ascospores of *Pyrenophora* type and thus represent immature perithecia which under favourable conditions may produce normal perithecia.

VI. Parasitism.

The leaves and leaf-sheaths of young and grown-up wheat plants were inoculated several times in the following manner :—

1. Small pieces of naturally infected wheat leaves showing plenty of spores were washed in mercuric perchloride solution (1:1000) for five minutes and then with several changes of distilled water, and placed on leaves of wheat plants. The inoculated plants were placed in glass moist chambers for forty-eight hours and later on kept outside. Infection experiments in this way were carried out several times.

2. Mycelium with agar was incubated in moist chambers, and after two days all the spores formed were washed in a watch glass. A drop of spore suspension was placed on a large number of leaves of several plants, and all the inoculated plants were incubated in a glass moist chamber for forty-eight hours and then placed outside in the open.

3. Mycelium from a pure culture was placed on leaves and leaf-sheaths either on the upper or the under surface of several wheat plants both young and grown-up, and all the pots were kept in glass moist chambers for forty-eight hours and later removed outside.

In all cases controls were kept and were intact. The infection was cent. per cent. in all cases.

The first discoloration of the inoculated portion was noticed after twenty-four hours, and later on the inoculated spots developed symptoms as in nature and produced conidiophores and conidia in the central dead portion. Several re-isolations were made and they were found to be identical with the original culture. In old inoculated plants sclerotial bodies or immature perithecia were formed with conidiophores and conidia on them as in sterilized straws.

VII. Cross inoculation.

In order to see whether this wheat fungus is identical morphologically with the form occurring on couch grass which it resembles, a series of inoculation experiments were carried out on couch grass (*Agropyron repens*) leaves, leaf-sheaths and stalks in the usual manner. In all cases it was found that the wheat fungus was capable of infecting couch grass and producing fructification on the inoculated plants. Further, it was observed that the disease symptoms produced on couch grass were similar to those described by Drechsler [1923] for *H. tritici-repentis* on *Agropyron repens*.

VIII. Taxonomy.

The fungus under consideration does not resemble morphologically any species of *Helminthosporium* recorded on wheat from various parts of the world except *H. tritici-vulgaris* described by Nisikado [1929] from Japan, but differs from it in certain morphological aspects. The description of this fungus also does not coincide with that of any other species recorded on any cereal or grass except *H. tritici-repentis* Died. recorded on *Agropyron repens* [1923].

According to Nisikado [1929,2] spores of *H. tritici-vulgaris* are yellowish brown to light yellowish brown and sometimes curved, while in this fungus spores are mostly subhyaline, and curved spores are very rare. Spores of *H. tritici-vulgaris* do not show a shape suggesting the tip aspect of a snake's head in outline as noticed in the species under consideration. *H. tritici-vulgaris* has been found to form conidia and conidiophores in culture, while this fungus does not form any and remains sterile or forms abnormal spores. Nisikado has not mentioned the formation of any sclerotial bodies in culture or perithecia but here sclerotia-like bodies are formed in very large numbers on all media tried. These do not develop further on agar media but on straw culture or on decaying tissue they further develop and form immature perithecia of *Pyrenophora* type with abundant conidiophores and conidia on their surface and in rare cases few asci and ascospores are also noticed. The development of asci and ascospores is checked by the formation of conidia.

In length, width and septation the fungus under consideration agrees with both *H. tritici-vulgaris* and *H. tritici-repentis* but it agrees very closely with *H. tritici-repentis* in spore shape, the proximal portion of the basal end of which tapers abruptly in the manner of a cone suggesting the appearance of the head of a snake. In addition to the shape of the conidia it agrees in colour, both being typically subhyaline. Further, this wheat fungus is capable of infecting *Agropyron repens* and of producing symptoms similar to those produced by *H. tritici-repentis*. Thus the writer is inclined to call it a strain of *H. tritici-repentis*.

H. tritici-repentis was first described by Diedicke [1902] as a biologic form of *H. gramineum* Rabh. and later on [1903] established as an independent species quite distinct from the allied species with subhyaline spores such as *H. gramineum*, *H. teres*, *H. Bromi* and *H. avenae*. It has never before been recorded on wheat and this is the first record of its occurrence on this host.

IX. Summary.

1. *Helminthosporium sativum* P. K. & B., *H. bicolor* Mitra, *H. halodes* Drech. var. *tritici* Mitra and *H. tritici-repentis* Died. have been recorded on wheat from India.

2. The fungus described here in detail is a strain of *H. tritici-repentis* Died. commonly found on *Agropyron repens*.

3. It does a good deal of damage in some years and usually occurs with *H. sativum* from which it cannot be easily distinguished by external symptoms.

4. The parasitism of the fungus is proved by several inoculation experiments.

5. This is the first record of its occurrence on wheat.

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WILT DISEASE OF *CROTALARIA JUNCEA* LINN. (SANN-HEMP).

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I. INTRODUCTION.

Crotalaria juncea (sann-hemp) is grown widely in India and is used as a green-manure, for fibre and as fodder. It is a crop of great economic importance. During the last three or four years sann-hemp plants have been found to suffer from wilt to a great extent in Pusa and its neighbourhood and the disease causes considerable damage in certain areas especially when the crop is grown for seed production and fibre. In 1931-32 the loss was estimated to be sixty per cent. in certain fields and it was about twenty-three per cent. during 1932-33.

The wilt disease of sann-hemp has also been recorded from the Madras and Bombay Presidencies and appears to be widely distributed throughout India wherever the crop is grown.

II. Historical.

The disease has been recorded from several countries. Vincens [1921] reported the occurrence of a wilt affecting *C. juncea* and *C. usaramoensis* L. from Tonkin. He described it as a collar disease similar to the wilt of pigeon-pea (*Cajanus indicus*) and cotton. Vincens isolated several fungi but he found a Fusarium to be constantly present closely allied to *Fusarium udum* Butl. (=*F. vasinfectum* Atk.) but failed to prove the pathogenicity of the fungus. Van Hall [1925] recorded wilt of *C. usaramoensis* from Dutch East Indies. Briant and Martyn [1929] recorded it from Trinidad and found it to resemble *Fusarium vasinfectum* Atk. from pigeon-pea. On the Manjri Farm (Bombay) Uppal [1931] found that the cause of wilting of sann-hemp was due to a Fusarium and obtained eighty-eight per cent. infection in infected soil; plants in a green house where the temperature was fairly high escaped the disease, while those in a cool green house suffered badly from wilt.

III. Symptoms.

The symptoms of the disease have been described by Briant and Martyn [1929] and also by Wright and Leach [1931] and agree closely with the symptoms as observed in Pusa. The following is a brief account of the symptoms as observed by the writer.

Generally the leaves of healthy sann-hemp plants turn upwards so that the under-surface is exposed, but in wilted plants the leaves gradually wither, droop or hang down and later on turn brown and ultimately within a day or two the plants die. Usually the whole plant wilts but when branching has taken place, it is confined sometimes to particular branches. When partial wilting occurs if the infected branches which have wilted are removed, it takes several days for the whole plant to wilt. In grown up plants the wilting parts droop at the tip and defoliation starts. After the plant has died, the sporodochia of the fungus with a pinkish tinge are produced on the dead stem or dead portion of the stem where the infection is confined to one side. This discoloration is sometimes noticed before the plant has actually wilted.

The discoloration of the tissue could be traced to the main tap root or lateral roots. In the early stages the fungus is confined to the lateral roots, especially the tip portion showing that the origin of wilt is from the roots. The fungus which has a saprophytic habit in the soil attacks the plants by roots or lateral roots or even through the cracking in the basal portion of the stem. The bark is covered with a pinkish mass of fungal growth and the pinkish colour is due to the presence of fructification of Fusarium producing both micro and macro-conidia. The spores are capable of infecting a growing crop.

The fungus can be noticed on pods and in many cases on the seeds in diseased pods. Wilted plants have been found to produce a large percentage of immature seeds. These seeds have the infection and when germinable are capable of disseminating the fungus. Mature and viable seeds seldom have the spores of this Fusarium but sound seeds may become infected at the time of threshing. The disease in the field generally occurs in patches and gradually spreads to the surrounding plants.

When the wilt is caused by *Rhizoctonia solani*, blackish discoloration of the bark from the base upwards and on the roots takes place with no pinkish discoloration of the bark. The tissues in such plants show the typical type of Rhizoctonia mycelium.

In the case of plants infected with *Neocosmospora vasinfecta*, the fungus forms salmon coloured fructification on the bark on the lower parts of the stem and also on the main root. In such plants neither the Rhizoctonia nor the Fusarium is present.

IV. Causal organisms and methods of isolation.

A large number of isolations was made from wilted sann-hemp plants in the following manner:—Small pieces of root or stem were taken from freshly wilted plants and sections were cut to see the presence of mycelium in the wood. The pieces were shorn of bark, treated with mercuric chloride (1:1000) for ten minutes and then washed in several changes of sterilised distilled water; they were then transferred to Brown's synthetic agar either in Petri dishes or test tube slants. It took some days for the mycelium to emerge from the cut ends and in several cases even fifteen days to make its appearance. The most common fungus isolated was a Fusarium which agreed in morphological characters with *Fusarium vasinfectum* from pigeon-pea and produced micro- and macro-conidia in abundance and chalamydospores later on and had a salmon pink colour as on the bark of the wilted plants. In order to see whether the Fusarium present on seeds is the same as that isolated from roots, seeds of sann-hemp were well shaken in water, centrifuged and cultures obtained. The isolated Fusarium was found to be similar to the one got from the roots. In addition to Fusarium, a species of Rhizoctonia and a species of Neocosmospora were also isolated. Though Fusarium was the most common one, Rhizoctonia sp. and Neocosmospora sp. were also fairly present and isolated several times. Rhizoctonia sp. was identified as *R. solani* Kühn. and agreed in cultural characters with the strain from piper-betel, chilli and other hosts. The species of Neocosmospora very closely resembled *Neocosmospora vasinfecta* Smith recorded from several hosts such as pigeon-pea, gram, cotton, etc. In cultural characters, colour formation and zonation it resembled the one described

by Butler [1910]. About two per cent. of the isolations gave rise to Neocosmospora and Rhizoctonia. In several cases Fusarium and Rhizoctonia were found to be associated.

Besides, a large number of wilted plants were examined microscopically and Fusarium was found to be present in the vascular tissue of roots, root-lets and stem. The xylem vessels of the roots were found to be plugged by masses of hyphae, offshoots from which in several cases extended into the parenchyma of the central cylinder and the cortex. The characteristic mycelium of *Rhizoctonia solani* was also found in about two per cent. of the plants both in the vascular tissue and the cortical region, sometimes alone and sometimes associated with Fusarium. In several wilted plants with characteristic pink colour, fructification of *Neocosmospora vasinfecta* Smith on the bark at the base of the stem and roots was noticed and when a sterilised piece of infected tissue was incubated, it gave rise to Neocosmospora.

The conclusion drawn from the microscopic examination of wilted plants and also from the isolations made, therefore, is that the chief cause of wilt of sann-hemp is Fusarium and to a lesser extent *Rhizoctonia solani* and *Neocosmospora vasinfecta*. These three fungi were isolated from wilted sann-hemp plants and were purified and grown in mass culture on sterilised rice and straw. Wilted material of sann-hemp and pigeon-pea was also collected and stored for soil infection. This was done with the following objects in view—

(*i*) to determine the pathogenicity of Fusarium in producing wilt of sann-hemp ;

(*ii*) to determine the pathogenicity of *Rhizoctonia solani* and *Neocosmospora vasinfecta* Smith, that is, whether these two fungi which are often associated with sann-hemp wilt have a saprophytic existence and appear only after the wilt is produced or whether they themselves are also capable of producing wilt ;

(*iii*) to determine the relation of the sann-hemp strain of Fusarium to the pigeon-pea, cotton and sesamum strains of Fusarium, that is, whether the wilt of one host can produce wilt in another.

V. Parasitism of the Fusarium.

In all infection experiments, the seed of sann-hemp was disinfected with 0·25 per cent. uspulun (universal) solution for thirty minutes before sowing. In all cases controls were kept. Every week on a fixed day all the dead plants were removed and each plant was examined to see if the death was due to Fusarium or due to any other cause.

Experiment 1.—Ten pots with soil were sterilised and the soil in these pots was infested with mass cultures grown in large flasks on sterilised straw and rice on

3rd August 1932. Disinfected seeds of sann-hemp were sown on 19th August 1932 and when the seedlings were four inches high, only five plants were allowed to remain in each pot. Thus the total number of plants was fifty. Before the end of November all the plants wilted and died and the tissue contained mycelium of Fusarium. Re-isolations gave cultures similar to the one used for infection.

Experiment 2.—An experiment similar to the previous one was repeated during 1933-34. Out of fifty-six plants, forty-five died of Fusarium wilt, six of other causes and five remained healthy.

Experiment 3.—Eighty plants of sann-hemp during 1932-33 were grown in pots where soil was previously sterilised and infection of soil was done with wilted sann-hemp material of the previous year. During the season seventy-four died of Fusarium wilt and six of Rhizoctonia. Death due to *Rhizoctonia solani* was only in young plants. The presence of Rhizoctonia was due to the presence of this fungus in some of the wilted material used for soil infection.

Experiment 4.—Another experiment similar to Experiment 3 was repeated during 1933-34 and out of one hundred and eight plants ninety died of Fusarium wilt, two of Rhizoctonia wilt and thirteen had both Fusarium and Rhizoctonia mycelium.

Experiment 5.—In a plot in which sann-hemp or pigeon-pea was never grown for at least fifteen years the soil was heavily infected with sann-hemp wilted material from the previous year's crop. The lower parts of wilted plants were cut into six-inch pieces. These pieces were placed about a foot below the surface of the soil on 12th July 1932 and disinfected sann-hemp seeds were sown on 27th July 1932. All wilted plants were periodically removed and each individual one was microscopically examined. Out of a population of two hundred and eighty plants, one hundred and ninety-three died of Fusarium wilt, twenty-six died of Rhizoctonia and sixty-one remained healthy. The percentage of wilt due to Fusarium was sixty-nine and most of the plants died during November and December. All the deaths due to Rhizoctonia were in the early stages of the growth of the plant.

Experiment 6.—The above experiment was repeated during 1933-34 in a plot where sann-hemp was never grown previously and the soil was heavily infected as before with sann-hemp wilted material of the previous year on 2nd June 1933. Disinfected seeds of sann-hemp were sown on 14th June 1933. The total number of plants in this plot was five hundred and eighty-seven, out of which four hundred and eighty-nine died of Fusarium wilt, that is, 83·3 per cent., twenty-four died of Rhizoctonia wilt, that is, 4·1 per cent. and sixty-eight plants had mycelium of both Fusarium and Rhizoctonia, that is, 11·5 per cent. had mixed infection. The number of healthy plants left at the end of the season was only six.

The above experiments conducted during 1932-33 and 1933-34 both in sterilised soil in pots and in plots show clearly that *F. vasinfectum* is the main cause of the wilt and that *R. solani* also causes death but only during the early stages of the growth of the plant. Sometimes wilt is caused by the presence of both fungi associated together. The infection due to *R. solani* in the above experiments was due to the presence of the fungus in the wilted material used for infecting soil. Controls remained free from the disease.

VI. Parasitism of Rhizoctonia.

In the previous experiments it was found that a certain per cent. of dead plants showed the presence of Rhizoctonia in the tissue, and whenever isolations were made a pure culture of Rhizoctonia was obtained. With a view to determine whether *Rhizoctonia solani* is also capable of producing wilt and also to see whether its presence is not secondary in wilted plants, infection experiments were carried out with pure cultures of *Rhizoctonia solani*.

Experiment 7.—Pots with soil were sterilised and the soil was infested with a mass culture of *R. solani* cultivated on boiled rice in big flasks on 3rd August 1932 and disinfected sann-hemp seeds were sown. Altogether there were forty plants and before December 1932 thirty-eight died. Re-isolations were made in several cases and in all a pure culture of Rhizoctonia was obtained.

Experiment 8.—An experiment similar to No. 7 was repeated during 1933-34. There were altogether seventy-four plants, sixty of which died of Rhizoctonia infection and the fungus was re-isolated several times. In nine Fusarium was found. This was due to secondary infection caused by the splashing of rain. Five remained healthy. These experiments carried out with pure culture on sterilised soil show clearly that *Rhizoctonia solani* is capable of producing wilt.

VII. Parasitism of Neocosmospora.

It has been mentioned previously that pure cultures of *N. vasinfecta* were isolated several times from diseased plants.

N. vasinfecta has been recorded from pigeon-pea, cotton, cow-pea, gram and indigo and from several other hosts. According to Butler [1910] *Neocosmospora vasinfecta* could not produce wilt of pigeon-pea, cotton or indigo, but no infection experiments were carried out by him on sann-hemp or with sann-hemp strain on other hosts.

In order to see whether *N. vasinfecta* is capable of producing wilt of sann-hemp the following experiments were carried out.

Experiment 9.—Ten pots with soil were sterilised in a steam steriliser and the soil was thoroughly infected with a pure culture of *N. vasinfecta* on 3rd August 1932.

Disinfected seeds of sann-hemp were sown in these pots on 19th August 1933. Altogether there were forty plants. By the end of December thirty-seven died and an examination showed the presence of Neocosmospora fructification above the collar region. Several isolations were made and in all cases pure cultures were obtained.

Experiment 10.—An experiment similar to No. 9 was repeated during 1933-34. There were altogether seventy-three plants, fifty-nine wilted and had Neocosmospora mycelium in the tissue. Eleven had both Neocosmospora and Rhizoctonia and three remained healthy. Rhizoctonia infection here was due to the secondary infection from pots in which Rhizoctonia infection was given and the spread on account of splashing of rain water.

These two experiments show that Neocosmospora is capable of producing wilt.

From the above experiments it is clear that *F. vasinfectum* is the chief cause of wilt of sann-hemp in Pusa and that wilt is also caused to a smaller extent by *R. solani* and *N. vasinfecta*. *R. solani* generally produces wilt when the plants are young. It is also the case with pigeon-pea [McRae and Shaw, 1933]* in which *R. solani* produces wilt when the plants are young.

VIII. Cross inoculation experiments with sann-hemp Fusarium.

In order to see whether the sann-hemp-strain of *Fusarium vasinfectum* produces wilt in *Cajanus indicus*, cotton, *Sesamum indicum* L., the following experiments were carried out.

Experiment 11.—During 1932-33, eighty plants of sann-hemp and seventy-six plants of pigeon-pea were grown in pots with sterilised soil which was infected with sann-hemp Fusarium. Ninety-two per cent. of sann-hemp and sixty-four per cent. of pigeon-pea plants wilted. Six plants of sann-hemp got Rhizoctonia infection which was secondary.

Experiment 12.—The above experiment was repeated during 1933-34 and out of one hundred and eight sann-hemp plants ninety died of Fusarium wilt, two of Rhizoctonia and thirteen had both Fusarium and Rhizoctonia. There were one hundred and twenty-one plants of pigeon-pea, out of which ninety-four died of Fusarium wilt, one of Rhozoctonia and three had both Fusarium and Rhizoctonia mycelium. The results of infection experiments in pots are summarised in Table I giving deaths month by month.

* The type of pigeon-pea (*Cajanus indicus*) used in the various experiments referred to in this paper was Pusa Type 5 which has been reported by McRae and Shaw [1933] to be highly susceptible to the Fusarium wilt and was very kindly supplied by Dr. F. J. F. Shaw, Imperial Economic Botanist. The seed of sann—hemp used was local seed secured from the Imperial Agriculturist who had collected it from a field of sann-hemp where wilt was severe. All seeds were disinfected before sowing.

TABLE I.

Sann hemp Fusarium infection in sterilised soil in pots.

	1932-33				1933-34					
	Sann-hemp		Pigeon-pea		Sann-hemp			Pigeon-pea		
	Fusarium wilt	Rhizoctonia wilt	Fusarium wilt	Rhizoctonia wilt	Fusarium wilt	Rhizoctonia wilt	Both Fusarium and Rhizoctonia	Fusarium wilt	Rhizoctonia wilt	Both Fusarium and Rhizoctonia
July	..	..	..	..	12	2	..	..	..	..
August	..	..	..	..	38	..	6	3	..	..
September	..	5	..	..	24	..	2	29	..	..
October	18	..	..	..	7	..	..	52	..	..
November	29	1	12	..	9	..	5	7	..	2
December	22	..	14	..	..	..	..	..	1	1
January	2	..	10	..	..	..	..	3	..	..
February	3	..	13	..	..	..	..	..	..	..
Total deaths	74	6	49	..	90	2	13	94	1	3
Healthy plants left	*Nil*		27		3			23		

Experiment 13.—In a plot where sann-hemp and pigeon-pea were never grown, the soil was thoroughly infected with sann-hemp wilted material of the previous year on 2nd June 1933 and disinfected seeds of pigeon-pea and sann-hemp were sown in alternate rows, eight rows of sann-hemp and eight rows of pigeon-pea. During the season out of a population of five hundred and ninety-five plants of sann-hemp, four hundred and ninety-eight died of Fusarium wilt, twenty-six of Rhizoctonia and sixty-five had both Fusarium and Rhizoctonia. There were two hundred and eighty-eight plants of pigeon-pea and one hundred and eighty-seven died of Fusarium wilt and eight had both Rhizoctonia and Fusarium. The results are summarised in Table II and give the number of deaths month by month.

TABLE II.

Sann-hemp Fusarium infection in soil during 1933-34.

	Sann-hemp			Pigeon-pea		
	Fusarium wilt	Rhizoctonia wilt	Both Fusarium and Rhizoctonia	Fusarium wilt	Rhizoctonia wilt	Both Fusarium and Rhizoctonia
August	184	16	2	18	..	..
September	247	..	20	40	..	..
October	54	..	11	42	..	..
November	12	8	16	15	..	2
December	..	2	16	9	..	5
January	..	..	..	11	..	1
February	..	..	..	27	..	..
March	..	..	..	5	..	..
April	1	..	..	20	..	..
Total deaths	498	26	65	187	..	8
Healthy plants left		6			93	

From Tables I and II it will be noticed that during 1933-34, wilting in sann-hemp started earlier as compared to 1932-33. This may perhaps be due to excessive rainfall during 1933. The rainfall (in inches) during 1932-33 and 1933-34 was as follows :—

	June	July	August	September	October	November	December
1932	6·66	5·90	7·08	7·85	0·58	2·53	0·3
1933	8·25	20·1	10·61	11·21	2·21	0	0

Experiment 14.—Sterilised soil in pots was infected with sann-hemp wilt material of the previous year and forty plants of cotton (Dharwar susceptible to wilt type) and eighty plants of sesamum were grown. Neither cotton nor sesamum took infection.

All these experiments in sterilised soil and plots indicate that the sann-hemp strain of *Fusarium vasinfectum* is capable of producing wilt in pigeon-pea under Pusa conditions but cotton and sesamum are immune to its attack.

IX. CROSS INOCULATION EXPERIMENTS WITH PIGEON-PEA FUSARIUM.

With a view to see whether the pigeon-pea strain of Fusarium can produce wilt in sann hemp, cotton and sesamum, the following experiments were carried out :

Experiment 15.—Sterilised soil in pots was infected during 1932-33 with pigeon-pea wilt material collected from the previous year and disinfected seeds of pigeon-pea and sann-hemp were sown. Out of seventy-eight plants of pigeon-pea, seventy-two died of wilt and six remained healthy. There were sixty plants of sann-hemp and thirty-two wilted leaving twenty-eight healthy plants. Thus the percentage of wilt due to pigeon-pea Fusarium was ninety-two and fifty-three respectively in pigeon-pea and sann-hemp.

Experiment 16.—The above experiment was repeated during 1933-34 and out of one hundred and nine plants of pigeon-pea ninety-one died of Fusarium wilt, eleven of *Rhizoctonia solani* and seven had both Fusarium and Rhizoctonia. There were one hundred and two plants of sann-hemp, seventy-eight died of Fusarium wilt, nine of Rhizoctonia and eleven had both Fusarium and Rhizoctonia. These two experiments show clearly that pigeon-pea Fusarium is capable of producing wilt in sann-hemp. The results of these pot experiments are summarised in Table III.

TABLE III.

Pigeon-pea Fusarium infection in soil.

	1932-33				1933-34					
	Pigeon-pea		Sann-hemp		Pigeon-pea			Sann-hemp		
	Fusarium wilt	Rhizoctonia wilt	Fusarium wilt	Rhizoctonia wilt	Fusarium wilt	Rhizoctonia wilt	Both Fusarium and Rhizoctonia	Fusarium wilt	Rhizoctonia wilt	Both Fusarium and Rhizoctonia
July	..	..	..	..	19	2	..	..	2	..
August	..	..	..	..	43	9	6	16	..	2
September	1	..	..	..	24	..	1	44	..	2
October	8	..	..	..	4	..	..	16	..	1
November	31	..	8	..	1	..	..	2	7	6
December	16	..	1	..	..	..	..	..	..	..
January	5	..	6	..	..	..	..	..	..	..
February	11	..	17	..	..	..	..	..	..	..
Total deaths	72	..	32	..	91	11	7	78	9	11
Healthy plants left	6		28		*Nil*			4		

In order to test the infection under field conditions the following experiments were carried out:—

Experiment 17.—In an one-tenth acre plot very heavily and uniformly infected with pigeon-pea Fusarium, two hundred and twenty-eight plants of susceptible Dharwar cotton, two hundred and fifteen plants of susceptible pigeon-pea and two hundred and four plants of sann-hemp were grown in alternate rows during 1932-33 and at the time of harvest none of the cotton plants had died of wilt, while eighty-five per cent. of pigeon-pea and fifty-seven per cent. of sann-hemp had died of Fusarium wilt.

Experiment 18.—The above experiment was repeated during 1933-34 but, instead of cotton, sesamum was grown. There were four hundred and seventy-eight plants of pigeon-pea out of which four hundred and sixty-nine died of Fusarium wilt giving 98·6 per cent. deaths. Of four hundred and sixty-five plants of sann hemp, four hundred and forty-four wilted on account of pigeon-pea Fusarium, that is, 95·5 per cent. None of two hundred and four plants of sesamum wilted. The wilted plants in the above two experiments are shown in Table IV, month by month.

TABLE IV.

Pigeon-pea, sann-hemp, cotton and sesamum grown in a plot heavily infected with pigeon-pea Fusarium

	Pigeon-pea		Sann-hemp		Cotton	Sesamum
	1932-33	1933-34	1932-33	1933-34	1933	1934
June	..	1	..	..	..	..
July	..	..	..	..	..	..
August	0	12	..	49	..	..
September	6	45	3	262	..	..
October	27	164	7	117	..	..
November	48	134	32	12	..	..
December	28	82	42	1	..	..
January	27	11	28	..	..	..
February	26	19	24	3	..	..
March	21	1	8	..	..	..
Total deaths	183	469	116	444	..	..
Healthy plants left	32	9	88	21	228	204

The figures of Table IV are further summarised in Table V.

TABLE V.

Wilt due to pigeon-pea Fusarium in pigeon-pea, sann-hemp, cotton and sesamum.

	1932-33			1933-34		
	No. of plants	Number wilted	Percentage	No. of plants	Number wilted	Percentage
Pigeon-pea	215	183	85	478	469	98·6
Sann-hemp	204	116	57	465	444	95·5
Cotton	228	*Nil*	*Nil*	..	..	..
Sesamum	..	..	..	204	*Nil*	*Nil*

The data obtained from the experiments in sterilised soil were confirmed under field conditions, that is, the pigeon-pea strain of Fusarium is capable of producing wilt in sann-hemp but not in cotton and sesamum.

Experiment 19.—In another experiment (*a*) sterilised soil in pots was infected with pigeon-pea wilted material obtained from the pigeon-pea plants which had died in the previous year of sann-hemp Fusarium. Of eighty-three plants of sann-hemp seventy wilted and ten had both Fusarium and Rhizoctonia. No plant survived. There were eighty plants of pigeon-pea, forty-seven died of Fusarium wilt, two of Rhizoctonia and thirty-three had both Fusarium and Rhizoctonia. None survived.

(*b*) Sterilised soil was infected with sann-hemp wilted material obtained from the sann-hemp plants which had died in the previous year of pigeon-pea wilt. There were fifty-three plants of sann-hemp, forty-six wilted of Fusarium, seven had both Fusarium and Rhizoctonia. None survived. Out of fifty plants of pigeon-pea thirty-nine had Fusarium and eleven had both Fusarium and Rhizoctonia. None survived.

Experiment 20.—The soil in which young plants of pigeon-pea, sesamum and cotton were growing was infected with strains of *F. vasinfectum* from pigeon-pea and cotton, but only pigeon-pea plants became infected with the Fusarium strain of pigeon-pea, and cotton with the Fusarium strain of cotton.

—	Fusarium strain of pigeon-pea	Fusarium strain of cotton
Pigeon-pea	+	—
Sesamum	—	—
Cotton	—	+

From the monthly record of the wilted plants it appears that higher temperature favours wilt in sann hemp, while lower temperature and maturity favour in pigeon-pea.

X. Conclusion.

These experiments demonstrate that sann-hemp wilt and pigeon-pea wilt are caused by similar strains of *F. vasinfectum* Atk. under Pusa conditions. They are similar to the fungus causing wilt of pigeon-pea described by Butler [1910] who first called it *F. udum* Butl., but later on considered it to be a biologic strain of *F. vasinfectum* [Butler, 1926]. Wilt is also caused to a lesser extent by *Rhizoctonia solani* and *Neocosmospora vasinfecta*. The cross inoculation experiments carried out both in sterilised soil and plots show that the sann-hemp strain of Fusarium can infect pigeon-pea, but not cotton and sesamum, and the pigeon-pea strain can infect sann-hemp but not cotton and sesamum.

According to Briant and Martyn [1929] and Thorold [1931] the sann-hemp strain of Fusarium cannot infect pigeon-pea, cow-pea, Bengal beans, sword bean, but there is no evidence of any special experiment having been carried out with all these hosts in a very heavily infected soil. They also maintain that pigeon-pea grown in plots previously occupied by sann-hemp took no infection, but whether the soil was heavily and uniformly infected or not is not known or whether the pigeon-pea grown was a susceptible or a resistant type. Their failure to take infection may perhaps be due to their having a different strain of sann-hemp Fusarium to the one obtained in Pusa or due to difference in soil and climatic conditions. This is supported by the fact that pigeon-pea Pusa Type 80 [McRae and Shaw, 1933] which is very resistant to wilt in Pusa is susceptible to wilt in Poona and Gokak.

Sann-hemp is very often grown in Bihar on land on which pigeon-pea had been grown in previous years. As both these crops are liable to be attacked by wilt disease, the danger of growing sann-hemp is much too obvious. Further, in any rotation that may be recommended for the control of pigeon-pea wilt, the sann-hemp crop should always be avoided. Wilt diseases are usually controlled by breeding disease resistant types. So in any programme involving such studies, the fact that not only *F. vasinfectum*, but *R. solani* and *N. vasinfecta* do cause wilt in a greater or lesser degree must be kept in mind.

There is a well marked cold season, December to February, when the mean temperature remains in the neighbourhood of 50°F. The monsoon period June to September is hot and damp and after the middle of October the temperature gradually falls till cold weather conditions set in by the middle of November. Sann-hemp and pigeon-pea are generally sown by the middle of June or the beginning of July, that is, when the monsoon starts. From July until the end of September when the temperature is very high Fusarium wilt is rare. From the second week of October when the temperature becomes cooler, wilt becomes manifest and increases as the

temperature lowers down. Wilt in sann-hemp generally starts much earlier than in pigeon-pea.

As a certain percentage of wilt of sann-hemp is caused by the fungus carried by seeds which get infection from diseased pods or at the time of threshing, it is advisable to disinfect the seed before sowing with some fungicides such as uspulun (universal) 0·25 per cent. for thirty minutes or with mercuric chloride (1 : 1000) for ten minutes.

XI. Summary.

1. The parasitism of a biologic strain of *Fusarium vasinfectum* Atk. causing wilt of sann-hemp has been established. *Rhizoctonia solani* Kühn and *Neocosmospora vasinfecta* Smith are also capable of producing wilt but to a minor extent. The infection experiments with the latter two fungi gave positive results.

2. Fusarium is noticed on pods and in many cases on the seeds in diseased pods and sound seeds get infection at the time of threshing. So it is better to disinfect seeds with some fungicide, such as uspulun or mercuric chloride.

3. The experiments carried out show that the pigeon-pea strain of *F. vasinfectum* can produce wilt in sann-hemp and *vice versa*, while the cotton strain of *F. vasinfectum* does not infect pigeon-pea and sann-hemp, nor do the pigeon-pea and sann-hemp strains infect cotton or sesamum.

4. The deaths due to *R. solani* and *N. vasinfecta* are mostly in the early stages of the growth of the plant and Fusarium wilt appears later in the season when the temperature lowers down.

5. Wilt in sann-hemp starts earlier, that is higher temperature favours wilt in sann-hemp while lower temperature and maturity in the case of pigeon-pea.

M. Mohd. Taslim's help in conducting the field experiments is appreciated very much.

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THE LIFE-HISTORY AND BIOLOGY OF *GALERUCELLA BIRMANICA* JAC. (COLEOPTERA, PHYTOPHAGA, CHRYSOMELIDAE, GALERUCINAE) AND THE EXTERNAL MORPHOLOGY OF LARVA AND PUPA, PART I.

BY

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(With Plates XLVII-L and four text-figures)

Introduction.

The life-history of *Galerucella birmanica* has been rather incompletely described by Lefroy [1908-1912] in which he gives it the name *Galerucella singhara*, which, as he says was only an adapted one.

This species has been recorded as a bad pest on the leaves of water-nut or *singhara* (*Trapa bispinosa*) from Burma, Bhamo; Bihar; Muzaffarpur, 16th November 1907, 30th December 1907, Central Provinces: Bhandara, Sondad, 3rd December 1912, 18th August 1906. Assam: Kalsi, Sakartala, November 1907. In the United Provinces it has been recorded from Cawnpore. So far as the author is aware it has not been recorded from Aligarh District.

Finding certain differences in Lefroy's account of the life-history of this beetle and my own observations I took up the study of its life-history more completely, paying special attention to the egg-laying of the female, habit and behaviour of the adult and the larva.

Paterson [1930] has described the Chrysomelid larva as a whole and has laid special stress on the arrangements of tubercles and setae from the point of view of classification. In the sub-family Galerucinae he has studied nine species and from his observations it is evident that there is a great resemblance as regards form, structure, arrangement of tubercles and setae among the larvae of allied forms. In studying the chaetotaxy and the arrangement of tubercles, I have followed his plan of the hypothetical type.

Galerucella birmanica is a pest of great economic importance as *singhara* forms a staple diet of the inhabitants of many poor villages, and as very little work has been done on the anatomical and morphological side of this sub-family, I propose in subsequent papers to publish the results of my studies in this species. Paterson in his paper has not dealt with the morphology of the larva of *Galerucella birmanica*

but my studies of the external morphology of this larva confirm to a great extent his observations.

Material and method.

The larvae in all their stages of growth were collected from the village Malhaoka-nagla in the vicinity of Aligarh and kept in the laboratory under ideal field-conditions with but little variation in temperature and humidity. The adults and eggs were also collected in large numbers from the same locality and placed in similar conditions. The material at my disposal being plenty, I could make extensive observations both in the field and the laboratory.

In the laboratory the living animals and the eggs were kept in earthen pots, filled with water, and some soil at the bottom, the water-nut plants were placed in them and covered with *mosquito-netting* cloth.

For the study of the external morphology of larvae both fresh and preserved materials were used, but it was found that the larvae fixed in Carnoy and Boullion's fluids were ideal for the study of chætotaxy and other external features. The dissection of external parts was carried on by placing the preserved and freshly killed larvae of all the three stages in caustic potash from fifteen to thirty minutes, thoroughly washing them with water and then dissecting them under the high power of a binocular. After dissection and completely removing the muscles from the subsequent parts they were placed in glacial acetic acid from three to four hours, transferred to absolute alcohol and kept therein from three to two hours, in which period two or three baths of alcohol were given, then placing them in clove oil from one to three hours they were finally mounted in xylol-balsam. Staining of the chitinous parts was found to be of little value as the chitin nearly retained its original colour if placed in caustic potash only for a short time which made the structures quite visible.

The adults are of a dark reddish brown colour representing no external difference in the two sexes except for the fact that the females are slightly darker in colour than the males. With little power of flight and not very swift in their movements, it is a matter of no difficulty to capture them by hand.

The time of cultivation of singhara *in the Aligarh district and its relation with the emergence of the pest from hibernation.*

By the end of December the *singhara* plantation is nearly over and the beetles disappear. The ripe *singhara* fruits are then placed in earthen pots whose mouths are closed after filling them up with water, and allowed to remain for three months. By the end of March or beginning of April the *singharas* are taken out of these pots and burried in mud a knee deep. The ditches being flooded up with water. By the middle of May or the beginning of June the seedlings come out and the young leaves float over the surface of water. These plants multiply rapidly by

sending out their roots and shoots, and if allowed to remain for a month will cover the entire small tank. It is at particular spots and places where previously the *singhara* plants existed that these young seedlings are grown. These plants are then cut down from their roots and sold to other cultivators of *singhara* who cannot afford to grow the young seedlings. These young plants are then burried in soil under water by means of their roots and begin to multiply by vegetative means.

Through a careful and thorough search by the sides of ponds and tanks, at the period when the *singhara* plantation was over, I could find a few beetles hibernating in the cracks which were formed by the sides of tanks as a result of the drying up of water. These places are sufficiently moist. These beetles will go as deep as the cracks would allow them, being themselves incapable of burrowing. They remain in this condition for nearly five months without taking any food. By trying various plants, which remain green throughout the year at the sides of ponds I came to the definite conclusion that there is no alternative host. At the time when these beetles were most active as well as during the hibernation period and also just before and after hibernation I placed them in cages, with all these plants which grow in the vicinity of tanks and inside the water ponds together with the *singhara* plants and found that neither they took anything nor laid eggs but ultimately died away. While under the same conditions they thrived very well on *singhara* plants.

When the hibernation period comes nearer and nearer the amount of fat, in the body, which is of light reddish brown colour, increases and is nearly completely used up in the resting period. When the beetles emerge after hibernation they possess a much darker colour. By intensive observations in the field as well as in the laboratory it was found that warm moist conditions induce the beetles to increased activity while cold and dry atmosphere leads to lesser and lesser activity and finally to hibernation, if a suitable place be found, otherwise death follows.

The attack of the pest and the method of dispersal.—As has been previously mentioned the seedlings come out by the middle of May or the beginning of June. The beetles that survive the draught and remain in the vicinity of these young plants come out nearly at the same time generally in the early hours of morning attracted by the scent of leaves and begin to feed at first on the plants near the border of tank and assemble together under the natural instinct for copulation, as there is an abundance of food. After copulation they lay eggs and retire in the grass by the side of tank. They have got an instinct of assembling together even when they are few and dispressing when they increase greatly in number. The eggs are laid in succession, the larvae hatch out and within a few days the number is sufficiently increased.

As already said elsewhere these creatures are incapable of sustained flight. But it was found that in the locality of Aligarh, with the exception of one place, wherever there was *singhara* plantation there was an attack of this much dreaded pest. The chief source of dispersal of this pest is man. The wind and the flight of the insect itself are but of little avail. It is strange that the cultivators of *singhara* believe that this insect can travel hundreds of miles in one night by its flight aided by the currents of wind and wherever there is *singhara* this pest will inevitably reach there. This belief has entered into their minds on account of ignorance, as they could imagine of no other way in which the pest could reach from one place to another. In fact the dispersal is effected when the young plants are exported from one place to another, as the cultivators do not take much care to disinfect the young plants (which they buy and on which there is frequently an attack of the pest) from all the stages of this beetle. They are generally careful to destroy second and third stage larvae and the adults, but the eggs and first stage larvae generally escape detection. If a single cluster of eggs is left in a hundred freshly bought plants it is sufficient to become a pest within a course of time. In this way several clusters of eggs and many first stage larvae which are very inconspicuous objects are carried away with the plants to other localities where these plants are sown. As aforesaid there was one particular pond of *singharas* in Aligarh which was free from attack. By investigations, I was informed by the owner of this plantation that this was the first time that the *singharas* were sown in that tank, and that he had not bought the young plants from any other locality but had sown the fruits of *singharas* there. This fact definitely proves that the dispersal of the pest is entirely through the young plants which are transported from one place to another. The Agricultural Department of United Provinces should take the matter seriously and see that all the exported plants are free from the attack of the pest or have been properly disinfected.

The nature and the amount of loss.

The adults as well as the larvae feed upon the epidermis of *singhara* leaves. As is the case with nearly all the insects, the adults cause but little harm. The first and the second stage larvae are not so detrimental as are the third stage larvae. By eating away the young leaves by means of their blunt mandibles, they retard the growth of the plants, on account of which the production of flowers and subsequently the formation of fruit is much reduced. The cultivators of *singhara* kill this pest daily in large quantities at all the stages of its development and growth. In this way they keep it in check. In spite of killing the pest daily there is a loss of at least 40 per cent. On the other hand if the pest is allowed to multiply freely, it will, within a short time, destroy the entire plantation. Neither the adults nor the larvae attack the lower surface of leaves.

COPULATION AND THE EGG-LAYING HABIT.

Prior to copulation the adults assemble together and by the touch of the antennae recognize their mates. The male then mounts upon the back of the female which remains still if it wants to copulate, otherwise it does not allow the male to copulate by raising its hind and middle legs which is a sort of warning for the male. As soon as this signal is given the male moves away swiftly in search of some other mate. The female, after copulating once does not couplate again, unless it has laid all its eggs. The male copulates several times without any rest. During the act of copulation the male plays the most active part. When it rides over the female it holds it firmly at the prothorax by means of its forelegs and at the mesothorax by the middle legs. The hind legs are of no assistance. The long and pointed aedeagus is thrust into the female genital aperture. Both the male and female keep their antennae in constant motion and remain still for some time, the female then moves slowly from one place to another with the male on her back which occasionally moves the female vigorously from side to side. The copulation lasts from one and a half hour to five hours. It is the female which takes an active part in throwing the male off her back, by forcing him to loosen its grip with the aid of her fore and hind legs. The male after great struggle is forced to alight from the back of the female. Just after getting rid of the male the female moves here and there in search of some suitable leaf for laying her eggs. The female during her wanderings, occasionally, stops at certain leaves, presses her abdomen by means of the middle and hind pairs of legs, with her head kept downwards and the antennae cast below. Again it moves and repeats the same process several times. The egg-laying begins from two to four hours after copulation. Eggs being always laid on the upper surface of leaves in one or generally several clusters. The female after copulation has got the tendency to move away as far as possible from the place where the male has left her, and has been seen wandering alone. It generally deposits its eggs on the leaves which are most healthy and free from attack. During the act of laying the eggs, she raises her abdomen up with the head and prothorax bent downwards, and presses it on the sides from forwards backwards by its middle and hind tarsi and femori. It then lowers it down, deposits the egg, moves a little and goes on repeating the process; in this way a cluster of eggs is formed. A female may lay her eggs at one spot forming a big cluster or at several places on the same or other leaves forming in this way small clusters of from five to eight eggs. A single female at one time lays from ten to thirty eggs and during her lifetime lays thirty to hundred eggs.

The eggs.—The eggs are rounded, light reddish brown in colour when freshly laid, but gradually assume a darker colour, and when the time of hatching comes very near the upper surface becomes darker still. They are of uniform size measur-

ing from 0·4 mm. to 0·5 mm. in diameter. The freshly laid eggs are sufficiently soft so that when they come in contact with one another they become attached together as well as to the leaf by their bases quite firmly. Every egg is provided with an epichorion and chorion, the former is finely sculptured into hexagonal areas which feature is visible even in freshly laid eggs under the high power of a binocular and still more prominent in sections of fixed eggs. On the other hand Lefroy describes the eggs as smooth and not in actual contact with one another. The eggs are hatched from six to nine days according to the conditions of temperature. Very high and very low temperature, complete dryness and submergence under water kills the eggs. Slightly increased temperature accelerates the process of hatching.

Emergence of larva from the egg.—There are no special structures for the larva to make its way through the egg as is the case with so many chrysomelid larvae. The breaking of the shell takes place by the pressure of the anal foot of the larva and the passage is widened by cutting the aperture by means of mandibles. The larva just after emergence from the egg is yellowish brown in colour but soon the chitin darkens and it assumes dark grayish brown colour. From one to two hours it remains on the leaf without taking any food, and then very slowly moves about, begins to take food and goes to another leaf. In this way all the larvae emerged from a single cluster of eggs disperse to other leaves within a period of four to six hours.

The accompanying table indicates the duration of various stages right up from the egg to the adult stage.

	Date 1932		
Eggs laid	16th October	16th October	16th October
Eggs hatched	22nd ,,	23rd ,,	25th ,,
1st moult of larva	26th ,,	27th ,,	30th ,,
2nd ,, ,, ,, ...	29th ,,	31st ,,	4th November
3rd ,, ,, ,, ...	6th November	9th November	14th ,,
Larvae pupated	6th ,,	9th ,,	14th ,,
Imagines emerged	13th ,,	17th ,,	24th ,,

PLATE XLVII.

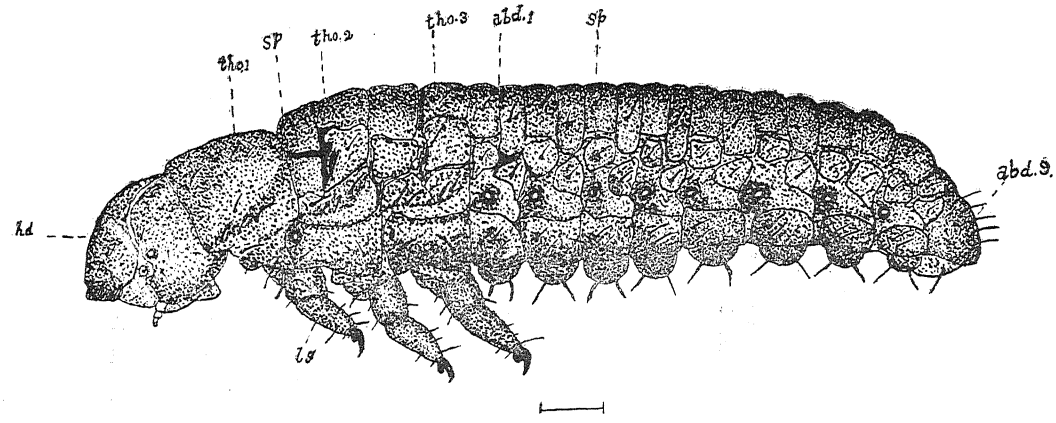


Fig. 1. The entire larva as seen from the lateral side—highly magnified.

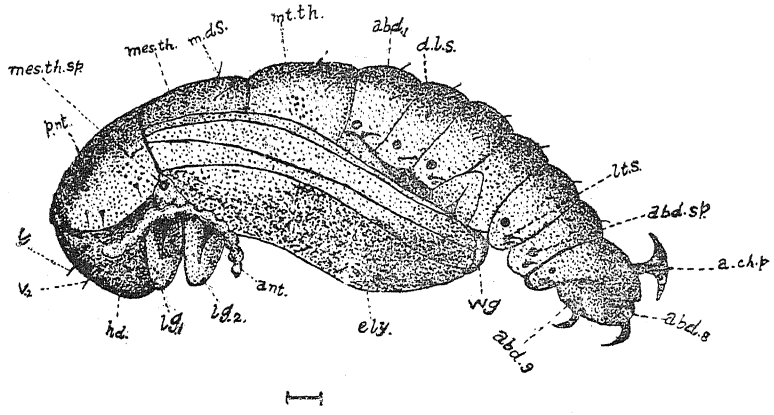


Fig. 2. The pupa—lateral view—highly magnified.

(For key to lettering see page 731.)

The duration of various stages :—

Eggs	six to nine days.
Larva	fifteen to twenty days.
Pupa	seven to ten days.
Length of first stage larva . . .	1 mm. to 1·5 mm.
„ „ second stage larva	4 mm. to 5 mm.
„ „ third „ „	7 mm. to 8·5 mm.
„ „ pupa	4·5 mm. to 5·2 mm.

Just before moulting the larva becomes quescent, assumes a very dark colour, and fixes itself to the surface of a leaf by means of its anal foot. Ecdysis takes place by the head of the larva being ruptured along the epicranial suture and along mid-dosal line of the promeso and metathorax which is in the same line as the stem of the epicranial arms. By raising the head and prothorax a pressure is exerted which causes this rupture. The head and thorax come out first, the larva assuming a globular form and then gradually by contraction and expansion the abdomen comes out. The entire process takes nearly two hours. The larva just after ecdysis is brownish yellow in colour but gradually very dark brownish gray colour is assumed. The cast out larval skins may be seen attached to the leaves. The larvae after casting their skins remain at the spot for sometime and then begin to disperse and attack fresh leaves.

The third moulting gives rise to pupa, the last larval skin remaining attached towards the posterior end to the hooks and spines. In captivity the imagines which emerged from the pupae did not copulate for at least a week.

External morphology of the larva.

For convenience' sake third stage larvae have been studied though there is no difference in the external features of the third and the first and second stage larvae.

The larva (Plate XLVII, fig. 1) is dark brownish gray in colour. It is cruciform, elongate and somewhat flattened on the ventral side, provided with a well developed head (hd.) which is held vertically and slightly retracted in the prothorax. In all, there are three thoracic (tho. 1-3) and ten abdominal segments (abd. 1-9) of which the ninth and tenth are greatly reduced. Each thoracic segments is provided with a pair of well developed short and stout legs (lg.). The prothorax and metathorax are subequal while the mesothorax is the smallest of the thoracic segments but broader than the abdominal segments. The abdominal segments taper gradually towards the posterior end.

Segmentally arranged transverse rows of tubercles bearing setae are present which in the prothorax and ninth abdominal segments coalesce to form a single tergal plate. A proleg or anal foot is present, at the apex of which the anus is situated, and is of great help in locomotion.

Head (*Plate XLVIII, figs. 3, 4*).—The head is bilaterally symmetrical and somewhat oval in outline. The epicranial suture is well defined and Y-shaped. It completely separates the vertex (v.) from the frons (f.). The stem of the epicranial suture or coronal suture (cs.) traverses nearly half of the distance between the attachment of clypeus and occipital foramen. The arms of the epicranial stem (epicranial arms: epa.) extends right up to the bases of the antennae (ant.). The frons (f.) is divided into two equal parts by a mid-dorsal longitudinal invagination (f. in.). This invagination provides for the attachment of the cephalic muscles. The fronto-clypeal suture (f. cs.) *i.e.*, the suture separating the frons from the clypeus is also strongly developed. As the mid-dorsal longitudinal invagination of frons and the strongly developed fronto-clypeal suture provide firm places for the attachment of muscles of the head, the tentorium has been completely obliterated. The fronto-clypeal suture extends laterally up to the dorsal articulation of mandibles.

The clypeus (cly.) (Plate XLIX, fig. 5) is differentiated into an anti (cly. 2) and a post-clypeus (cly. 1). The latter is more chitinized than the former, bears on either side a small process into which the socket on the dorsal side of the mandible fits and is provided with three pairs of setae (cl. 1-cl. 3), while the anti-clypeus is devoid of setae. The clypeo-labral suture (c. lr. s.) is well developed the middle part of which is rather strongly curved towards the frons. The free extremity of the labrum (lr.) is slightly fringed, otherwise it is entire. The labrum partly covers the mandibles and the mentum. The gena (gn.) is produced anteriorly into a sub-triangular hypo-stome (hyp.) bearing at its extremity a small socket for the condyle of the mandible (md.). A single dorso-lateral ocellus (oc.) on either side behind the antenna is present.

Arrangement of setae on various sclerites of the head.

On the vertex on either side of the coronal suture there are four primary setae (v1—v4) of which three are situated in one line along the arm of the epicranial suture, the fourth seta is situated just behind the third (counted from the middle of the head to the lateral region); moreover there is one very small seta on either side of the head towards the posterior extremity. Posterior to the ocellus there is a single small seta. On the frons there are three setae (F1—F3) on either side arranged in a triangle. Each gena is provided with a single long seta (gn. 1).

Appendages of the head.

The antennae (Plate XLIX, fig. 10) are situated dorsolaterally at the end of the epicranial arms. The base of each antenna is surrounded by an annular chitiniza-

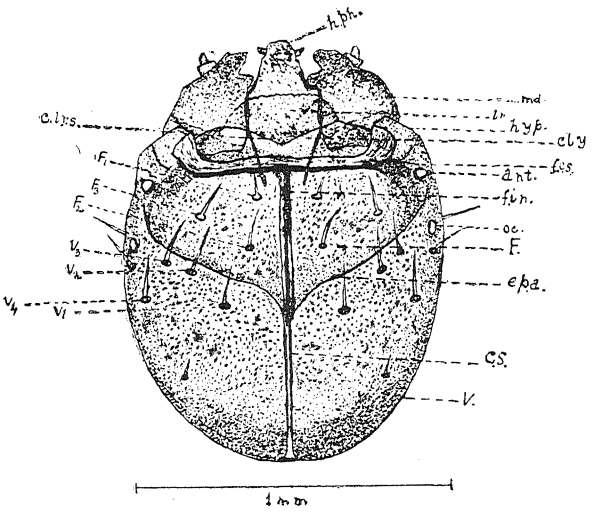


Fig. 3. Dorsal aspect of the head of larva with mandibles—highly magnified.

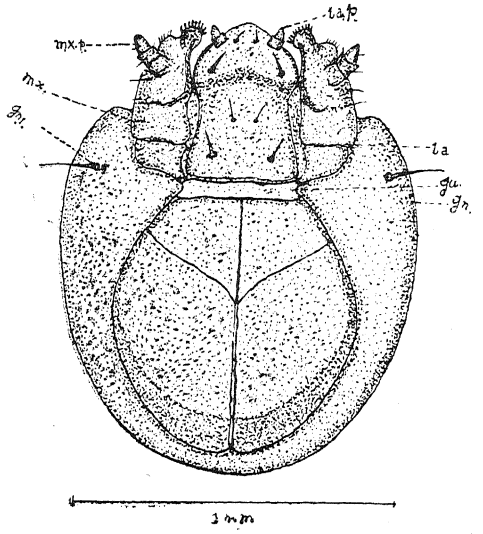


Fig. 4. Ventral aspect of the head of larva—mandibles removed—highly magnified.

(For key to lettering see page 731.)

PLATE XLIX.

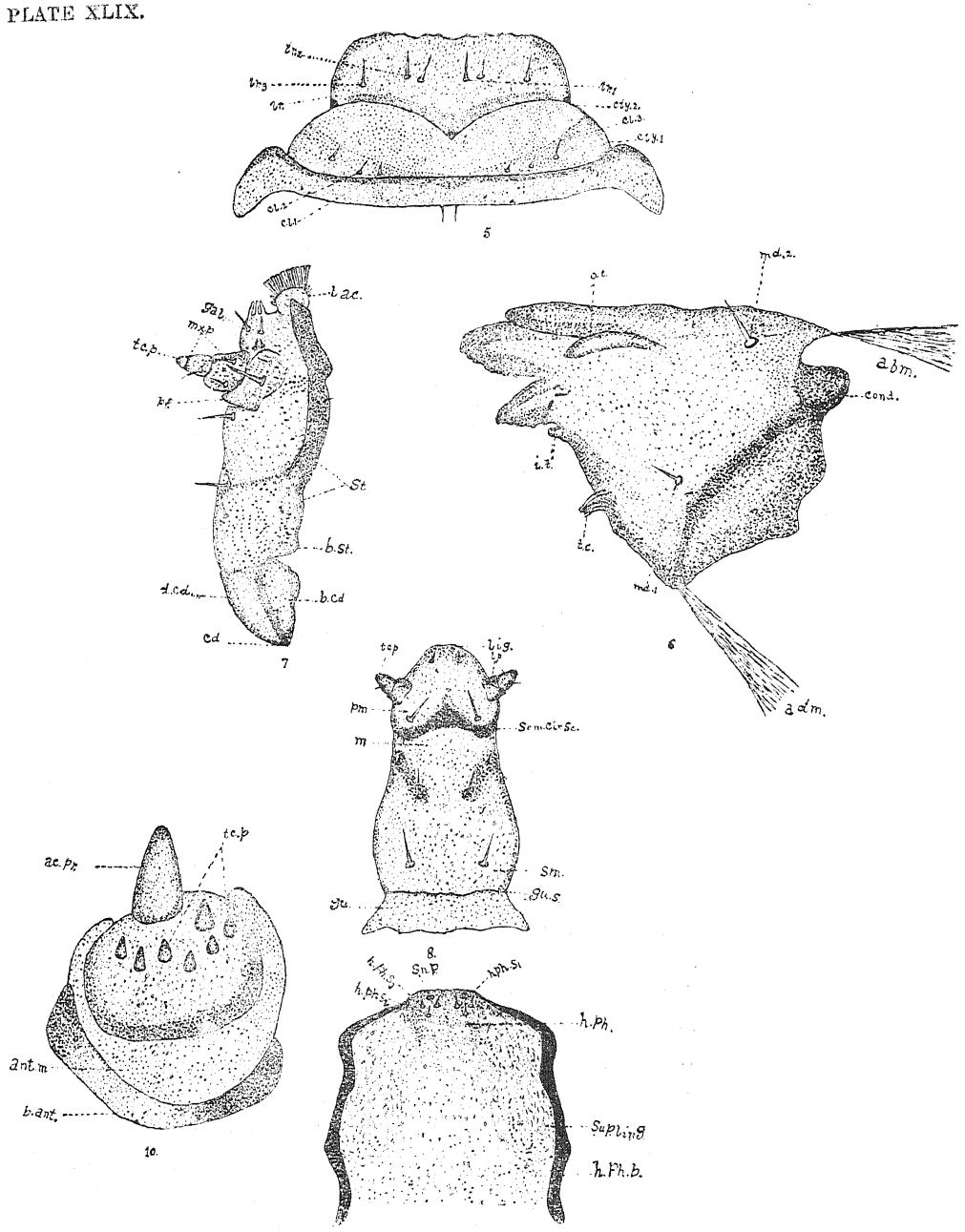


Fig. 5. Clypeus and labrum (×90)—Dorsal aspect.
Fig. 6. Mandibles (×90)—Ventral view.
Fig. 7. Maxilla of right side (×90)—Ventral view.
Fig. 8. Labium (×90)—Ventral view.
Fig. 9. Hypopharynx and Superlinguae (×90)—Dorsal view.
Fig. 10. Antenna (×405)—Dorso-lateral view.

(*For key to lettering see page* 731.)

tion termed baseantenna by Crampton. To this chitinization the antenna is attached by means of a membrane (ant. m.) which allows the antenna to be retracted or protruded, and also provides for considerable freedom of movement from side to side. The first and the third joints according to Paterson [1930] are absent. The second joint is provided with a conspicuous accessory process (ac. pr.) and the third joint is represented by seven tactile processes (tc. p.).

Mouth parts (Plate XLIX, figs. 5-9).—The labrum (ls. fig. 5) is provided with three conspicuous setae (lr. 1-lr. 3) on either side of its mid-dorsal line, its anterior region is slightly less chitinized than the posterior region. Anteriorly the epipharynx is attached to the antero-ventral extremity of the labrum, while posteriorly it is connected with the hypopharynx by means of a very thin membrane. On each side the epipharynx is provided with four large sensory patches of an irregular outline and from five to six rounded olfactory pores. Its anterior margin is provided with three small setae on either side.

The mandibles (fig. 6) articulate ventrally by means of a rounded condyle (cond.) to the hypostome and by means of a ginglymus to the post-clypeus. They are moved by powerful abductor and adductor muscles (abm., adm.). At the first sight the mandibles appear to be tridentate but a careful examination reveals the presence of five blunt teeth the inner margins of three of which are slightly serrated, the inner tooth (i.t.) being smaller than the outer tooth (o.t.). The outer tooth itself is much smaller than the three middle ones. In the middle of the inner margin of the mandible there is a tuft of cilia (t.c.). Two setae (md. 1, md. 2) are present on each mandible one at the outer and the other at the inner margin.

The maxilla (fig. 7) approaches very close to the typical insect maxilla. In primitive coleopterous larvae and in a long series of insects the cardo (cd.) is divided into eucardo and paracardo or basicardo and disticardo [Crampton 1921, 1923-24]. In the larvae of *Galerucella birmanica* the cardo is roughly triangular and divided into a basicardo (b. cd.) and a disticardo (d. cd.) but the line of demarcation of the two divisions of cardo is distinguishable only after careful examination. The cardo articulates ventrally with the head at the hypostomal prolongation of the gena. The stipes (st.) is a broad roughly rectangular sclerite and forms the main portion of the body of the maxilla. There is no clear differentiation of the stipes into eustipes and parastipes. The inner margin of the stipes which is attached to the mentum and the sub-mentum of labium is highly chitinized. This chitinization takes the form of a rod which is produced forward to support the lacinia. This chitinized bar may represent the parastipes of Crampton [1923-24]. The basistipes (b. st.) is slightly marked off from the distal portion of stipes, which, however, does not exhibit any differentiation into dististipes and eustipes. The stipes in all cases bear two long and two short setae.

The palpifer (pf.) which bears the two jointed maxillary palp is clearly differentiated from the stipes and possesses one long and one short seta. The first joint of the maxillary palp (mx. p.) is supressed [Paterson 1930], the second joint is broad and bears two moderately long setae. The third joint is rather narrow, bearing one moderately long seta and terminating into several very minute tactile processes (tc. p.). The galea (gal.) is situated on the palpifer at the inner margin of the maxillary palpus and bears seven setae.

The labium (fig. 8) has much departed from the primitive condition on account of great fusion having taken place in its various parts. The sub-mentum (sm.) and the mentum (m.) are slightly differentiated from one another by the presence of two lateral, rather inconspicuous chitinizations, marking the boundary of the former sclerite. The submentum is very broad and bears one pair of long setae. The mentum is a narrow subtriangular region just below the strongly chitinized semi-circular sclerite (sem. cir. sc.) and bears one proximal pair of long, and one distal pair of short setae. The latter sclerite has been termed the post-labial chitinization by Boving [1927]. Paterson thinks that it has been formed by the fusion and chitinization of the bases of palpigers. The palpigers have been completely fused together forming the greater part of prementum [Crampton, 1923]. The portion just above the semi-circular sclerite may be termed the prementum (p.m.) and bears a pair of long setae. The glossa and the paraglossa have also been completely fused to form the ligula (lig.). The labial palp (l. p.) is a two-jointed structure of which the first joint is broader than the second which is conical and bears at its tip several small sense papillae (tc. p.). The first joint of labial palp bears one short seta at its inner margin while the second joint possesses a similar seta at its outer margin. The ligula also bears a pair of very small setae. The gula (gu.) is represented by a very thin and narrow membrane connecting the submentum with the prosternum. It can easily be separated from the submentum, due to the presence of a gular suture (gu. s.).

The hypopharynx (fig. 9) is connected with the ligula by means of a suture and passes backwards dorsal to the labium forming the floor of the pharynx. Posteriorly the hypopharynx is connected with the membranous epiphrynx. On the sides it is strengthened by strong chitinized bars (h. ph. b.) which also enclose the superlinguae between them. The hypopharynx is a wide convex structure possessing at its distal extremity, in the mid-dorsal region, three pairs of short setae (h. ph. s1-s3). A single sensory pore (sn. p.) is situated in each half of the hypopharynx between the setae 1 and 3 and in front of seta 2.

The superlinguae (sup. ling.) have been regarded by Hansen [1893] as homologues of the Crustacean maxillulae and hence of appendicular nature and termed

PLATE L.

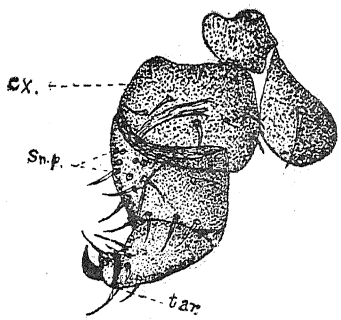


Fig. 11.

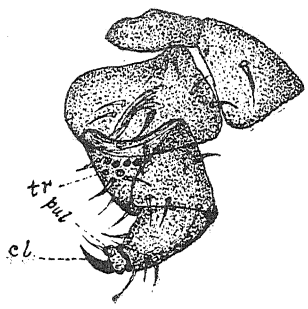


Fig. 12.

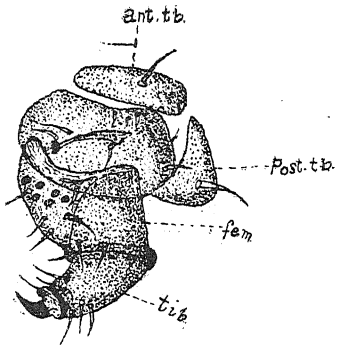


Fig. 13.

Fig. 11. Left prothoracic leg (×50)—dorsal view.
Fig. 12. Left mesothoracic leg (×50)—dorsal view.
Fig. 13. Left metathoracic leg (×50)—dorsal view.



Fig. 14. Eggs laid on the leaves (×80).

(*For key to lettering see page* 731.)

as maxillulae. Crampton [1921] is of opinion that they are in the same way associated with the hypopharynx as the paragnaths of Crustacea. In *Galerucella birmanica* they have been completely fused with the posterior margins of the hypopharynx from which they are distinguishable only by their more chitinized appearance and the presence on their dorsal surface of minute spinous setae.

The legs (*Plate L, figs. 11, 12, 13*).—There are three pairs of thoracic legs, which closely resemble each other in form, size and chaetotaxy. Each leg has got five joints, namely (counted from proximal to distal end)—coxa, trochanter, femur, tibia and tarsus. There is a single curved tarsal claw. By the side of the coxae of each leg there is an anterior (ant. tb.) and a posterior (post. tb.) tubercle. The distribution of setae on various joints of the leg is constant in all the individuals and may be of great importance in determining the species.

The coxa (cx.) is the broadest portion of the leg. It is very imperfectly divided into an anterior portion or coxa vera and a posterior portion or meron. It is provided with five long and three small setae.

The trochanter (tr.) or second joint of the leg is triangular and bears five setae and six sensory pores (sn. p.). It is the most membranous portion of the leg.

The femur (fem.) is thick and stout and bears four setae.

The tibia (tib.) is a narrow rather elongate structure provided with five conspicuous setae.

The tarsus (tar.) is represented by a small segment at the base of the single curved claw (cl.) and is provided with a single seta. The claw possesses a small pulvillus (pul.) at its base.

CHAETOTAXY AND THE ARRANGEMENT OF TUBERCLES.

The tubercles as defined by Paterson are chitinized, deeply pigmented, segmentally arranged areas which are furnished with one or several setae. The tubercles have been numbered from the mid-dorsal to the mid-ventral line.

The fusion of tubercles in the abdominal region is more prominent than in the thoracic segments except the prothorax.

In a typical segment *i.e.*, any of the first eight abdominal segments (Fig. 1) the tubercles and setae are arranged into a dorsal group I-IV extending from the mid-dorsal line of the body to the dorso-lateral tubercles; typically the dorso-lateral tubercles are represented by a pair of anterior and a pair of posterior tubercles V-VIII each bearing a seta but in *Galerucella birmanica* all the tubercles and setae have been obliterated or fused together and are represented by a single tubercle bearing seta VI. Projecting laterally from the dorso-lateral tubercle there is a single sub-spiracular tubercle provided with two setae—IX and

X—but in the mesothorax there are two distinct tubercles in the same position and the mesothoracic spiracle is located anterodorsally on the proximal tubercle. In the abdominal region the spiracles have migrated dorsally and the two sub-spiracular tubercles have been fused, but the setae IX and X are constantly retained. In the abdominal region ventral to the sub-spiracular tubercle there is a single pleural tubercle which is represented in the thoracic segments by a pair of sclerites at the bases of coxae. This paired origin of the pleural tubercles in the abdominal region is evident by the presence of a pair of setae XI and XII ; moreover it is further illustrated by the examination of the larvae which have just cast their skins, in which case they invariably possess a pair of pleural tubercles in the first abdominal segment, but, which consequently fuse together as the growth proceeds.

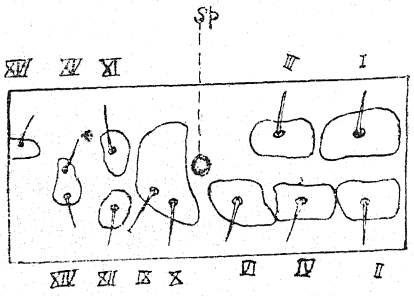


Fig. 1

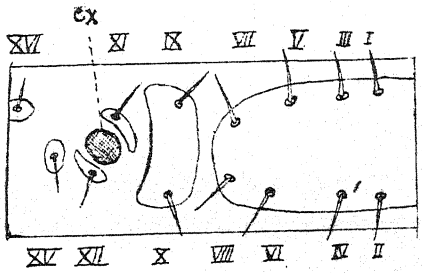


Fig. 2

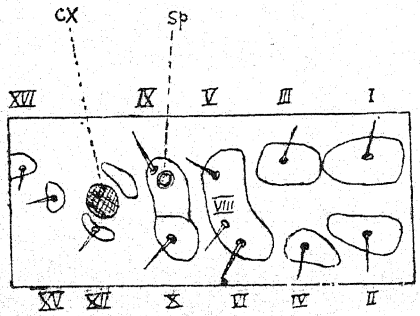


Fig. 3

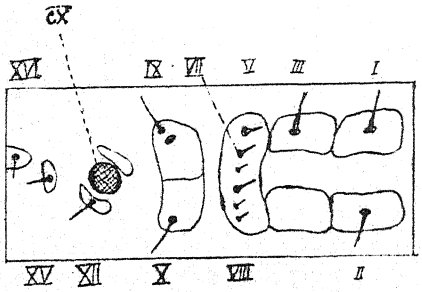


Fig. 4

Fig. 1. Setal plan of a typical abdominal segment (one of the anterior most segments). Fig. 2. Setal plan of prothorax. Fig. 3. Setal plan of mesothorax. Fig. 4. Setal plan of metathorax (at tubercle IX the closed spiracle is shown as a dark spot).

cx. = coxa
sp. = spiracle.

The tubercles on the sternum are arranged into a ventro-lateral group and a mid-ventral group *i.e.*, there is a pair of tubercles on either side of the mid-ventral tubercle, which is formed by the fusion of the two mid-ventral tubercles each bearing the seta XVI. The ventro-lateral tubercle of either side, in the abdominal region, is provided with setae XIV and XV, but in the thoracic region only seta XV is present. The mid-ventral group lies slightly in front of the ventro-lateral group.

In the prothorax (Fig. 2) the dorsal and the dorso-lateral tubercles have been fused together to form the single tergal plate which bears the typical I-VIII setae along its anterior and posterior margins. Setae IX and X are borne by a single tubercle which is present exactly in the same position as the sub-spiracular tubercle in the abdominal region. The two pleurites at the sides of the coxa are provided with setae XI and XII. The pronotum is divided mid-dorsally into two halves by a longitudinal line which is continuous anteriorly with the stem of the epicranial suture and posteriorly with similar line on the meso and meta-thorax. Along this line the rupture takes place at the time of larval ecdysis.

In mesothorax (Fig. 3) tubercle I tends to fuse with the tubercle III. The dorso-lateral tubercle bears the setae V, VI and VIII. Tubercles IX and X are distinct though slightly fused; they bear setae IX and X respectively. Seta XI is absent from the anterior pleural tubercle.

In the metathorax (Fig. 4) seta IV is absent though the corresponding tubercle is well defined. Seta VI is absent from the dorso-leteral tubercle. Between setae VII and VIII there is one microscopic seta. There are two more similar small setae at the side of the seta VIII towards the margin of the tubercle. In exactly the same position where the mesothoracic spiracle is located is a dark highly chitinized spot representing the vestigeal spiracle of this segment.

The ninth abdominal segment is represented by a single tergite bearing six setae on either side whose homology is rather obscure. This single tergite represents the fusion of the dorsal, dorso-lateral and sub-spiracular tubercles [Paterson, 1930]. The pleural tubercles have been suppressed. The ventro-lateral tubercle each bears the seta XV, seta XIV being suppressed. The mid-ventral tubercle typically bears one seta on either side.

The tenth abdominal segment is greatly reduced and represented by an inconspicuous sternite at the base of the proleg, bearing not a single seta, and two chitinizations by the sides of the same structure, which may represent the fused ventro-lateral and pleural tubercles.

Spiracles (*Figs. 1-4, Plate XLVII, fig. 1*).—The spiracles are slightly raised up from the general surface of the body. They are uniforous and surrounded by a

peritreme. There is one pair of spiracles in the mesothorax situated on the antero-dorsal tubercle of either half of the body. In the abdominal region the spiracles have migrated, dorsal and the antero-dorsal tubercle becomes sub-spiracular in position. There are in all, nine pairs of spiracles of which one pair is present in the meso-thorax and eight pairs from the first to the eighth abdominal segment.

Pupa (Plate XLVII, fig. 2).—The pupa measures from 3·5 mm. across the thorax and from 4·5 to 5·2 mm. in length. It is of ex-rate type characteristic of the order. The body is bright yellow just after the final larval ecdysis but gradually darkens assuming yellowish gray colour. The head (hd.) is strongly bent down and the tergum of the prothorax (pnt.) partly covers it. The antennae (ant.), legs and wings are closely approximated to the body and eyes are lightly pigmented. The prothorax and metathorax (mt. th.) are sub-equal in size, while the meso-thorax (mes. th.) is the smallest. A slight vestige of scutellum may be seen on the mesothorax. The abdominal segments are less broader than the thoracic ones and gradually taper towards the posterior extremity of the body. The elytrae (ely.) and wings (wg.) are curved ventro-laterally passing between the meso- and meta-thoracic legs nearly covering the latter but a short space is left in between the two pairs of opposite sides through which the metathoracic legs can be seen. The pro- and mesothoracic legs (lg. 1—lg. 2) are sharply bent towards the inner side of body at the femurotibial joint, their tarsi are bent in the opposide direction at the tibio-tarsus joint, hence lying in straight line with the body. The legs (including tarsi) reach up to the three-fourths of wings. The latter being slightly longer than the elytrae. When viewed from the dorsal side the elytra completely covers up the legs. The antennae which consist of eleven joints are curved, lying below the elytrae and from the third joint onwards above the femora and tibae of the fore and middle legs. The first two joints of the antenna are visible from the dorsal side. The terminal two joints project forwards from above the tibae. The wings and elytrae extend to the end of the fourth abdominal segment. There are three thoracic and nine abdominal segments (tho. 2, tho. 3 and abd. 1-abd. 9). All the setae are very inconspicuous. There are two pairs of setae (V1, V2) on the vertex, four setae on each half of the pronotum arranged in a semicircle. The other body segments including the meso and meta-thorax, the paired mid-dorsal setae (m. d. s.) are inconspicuous. There is a single dorso-lateral seta (d. l. s.) on either side of all the body segments, except the first thoracic and last abdominal. There is a single lateral seta (lt. s.) on either side of the body, which from the fifth to the ninth abdominal segments (both inclusive) becomes more and more chitinized as we proceed further, assuming hook like structure which is most prominent in the ninth abdominal segment and which has been termed spine by Paterson [1930]. By the arrangement and the gradual modification of these hooks and spines, I understand,

that the hooks and spines, whatever they may be called, are nothing but the modified setae which have assumed a curved shape, and, have become highly chitinized. The third, fourth and fifth abdominal segments have got two lateral setae on either side. The sixth abdominal segment is somewhat triangular in form. The seventh is highly modified, its distal extremity being roughly triangular. On the dorsal surface of this segment there is present a single anchor-shaped chitinous process (a. ch. p.) which seems to be modified mid-dorsal setae whose proximal and distal ends have been completely fused together in the middle line, and which distally are produced on either side assuming an anchor shape. The two arms of this process are curved and pointed, each arm may represent the distal part of a single dorsal seta.

The eighth and ninth abdominal segments are greatly reduced each bearing a curved spine on either side.

A pair of spiracles (mes. th. sp.) is present on the mesothorax. The abdominal segments, from the first to the eighth, each bears a pair of spiracles (abd. sp.). In the seventh and eighth segments they are greatly reduced. The ninth segment is devoid of spiracles. In the eighth and ninth abdominal segments the dorsal setae are not present.

Neither the larvae nor the pupae exhibit any sexual dimorphism.

Thanks to the unfailing efforts of Dr. Sir Syed Ross Masood, the Vice-Chancellor of the Muslim University, Aligarh, that the new science laboratories came into being and research work was started for the first time in the life of this institution. It was at the suggestion of Dr. M. B. Mirza, the Director, Zoological Laboratories, that I took up the present study. I am deeply indebted to him for his kind guidance, encouragement and the facilities which he provided to me in the course of this work. I take this opportunity to thank Mr. P. V. Isaac, the Officiating Imperial Entomologist, Imperial Institute of Agricultural Research, Pusa, the Director of that Institute and Rai Bahadur C. S. Misra for allowing me the use of literature and the museum freely. I shall be failing in my duty if I do not thank Mr. Maulik of the British Museum, London, Professor Dr. Bischoff and Professor Dr. Küntzel, Universität Giessen a.d. Lahn (Germany), for identification of this species.

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Key to the lettering of figures in Plates XLVII-L.

abd. 1-abd. 9	abdominal segments.
abd. sp.	abdominal spiracle.
ab. m.	abductor muscle.
ac. pr.	accessory process.
a. ch. p.	anchor shaped chitinous process.
a. d. m.	adductor muscle.
ant.	antenna.
ant. m.	antennal membrane.
ant. tb.	anterior tubercle.
b. ant.	base-antenna.
b. cd.	basicardo.
b. st.	basi-stipes.
cd.	cardo.
cl.	claw.
cl. 1-cl. 3	clypeal setae.
c.lr. s.	clypeo-labral suture.
cond.	condyle.
c. s.	coronal suture.
cx.	coxa.
d. cd.	disticardo.
d. l. s.	dorso-lateral seta.
ely.	elytra.
ep. a.	epicranial arm.
f.	frons.
F. 1-F. 3	frontol setae.
f. c. s.	fronto-clypeal suture.
fem.	femur.
f. in.	frontol invagination.
gal.	galea.
gn.	gena.
gn. 1	genal seta.
gu.	gula.
gu. s.	gular suture.
hd.	head.
h. ph.	hypopharynx.
h. ph. b.	chitinized bar of hypopharynx.
h. ph. s. 1-h. ph. s. 3	hypopharyngeal setae.
hyp.	hypostome.
i. t.	internal tooth.
la.	labium.
lac.	lacinia.
lg.	leg.
lg. 1-lg. 2	pro- and meso-thoracic legs.
lig.	ligula.
lp. lap.	labial palp.
lr.	labrum.
lr. 1-lr. 3	labral setae.
m.	mentum.
md.	mandible.

md. 1-md. 2	mandibular setae.
md. s. m.	mid-dorsal seta.
mes. th.	mesothorax.
mt. th.	metathorax.
mx.	maxilla.
mx. p.	maxillary palp.
oc.	ocellus.
o. t.	outer tooth.
pf.	palpifer.
p. m.	prementum.
p. nt.	pronotum.
post. tb.	posterior tubercle.
sem. cir. sc.	semicircular sclerite.
sm.	submentum.
sn. p.	sensory pore.
sp.	spiracle.
st.	stipes.
sup. ling.	superlinguae.
tar.	tarsus.
t. c.	tuft of cilia.
tc. p.	tactile processes.
tib.	tibia.
tho. 2-tho. 3	second and third thoracic segments.
tr.	trochanter.
V.	vertex.
v. 1-v. 4	setae on the vertex.
wg.	wing.

ON THE NATURE OF REACTIONS RESPONSIBLE FOR SOIL ACIDITY, PART III.

BY

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SPECIAL FEATURES OF COLLOIDAL ACIDS.

In a previous contribution [Mukherjee, Roychoudhury, Dasgupta and Chatterjee, 1932] evidence has been given to show that colloidal solutions of silicic acid behave in many respects as a strong acid, and in some other respects it has the character of a weak acid. Colloidal electrolytic systems have properties which require that they should be treated theoretically on a basis different from that of usual electrolytes [Mukherjee, 1933]. It is desirable at this stage to discuss the differences between these systems with reference to colloidal solutions of insoluble acid substances, as they elucidate the nature of the chemical reactions involving the colloidal acid complex in soil.

2. Rabinowitsch and Laskin [1928], and Pauli and Valko [1929], have observed that silicic acid sols possess the properties of a strong acid. Pauli and Valko [*loc. cit.*], prepared the sols by the hydrolysis of silico hexane and purified them by electrodialysis. The total acidity was measured by conductometric titration and the free acidity was calculated from the e. m. f. of the hydrogen electrode as also from the specific conductivity. Rabinowitsch and Laskin [*loc. cit.*] working with electrodialysed silicic acid sol, found that concordant pH values of the sol were obtained by both hydrogen and quinhydrone electrodes. They also estimated the total acidity of the sol by potentiometric and conductometric titrations. The dissociation constants of the various silicic acid sols, calculated from their potentiometric titration curves, were found to be of the order of 10^{-4} as compared to 10^{-9}, the approximate value of the first dissociation constant of silicic acid in true solution. The

percentage dissociation of the acid increases with the dilution unusually rapidly and at a dilution of 128 times of the sol it becomes 330. The degree of dissociation was calculated from the measured pH of different dilutions and the total acidity of the undiluted sol. This difference, between the truly dissolved acid and the colloidal acidoid electrolyte, is explained on the assumption that the colloidal particles contain the acid anhydride molecules, which are progressively hydrated as the dilution proceeds, and the dissociation of the hydrated molecules supplies fresh hydrogen ions. Another peculiarity observed by them was that on dilution of the silicic acid sol the specific conductivity increases and the pH decreases with time. After some hours a constant value of both the quantities are obtained. The pH—dilution curve of an arsenious sulphide sol was found [Rabinowitsch and Kargin, 1931] to be S-shaped in form and is explained as follows : At first a dissociation of the adsorbed hydrogen sulphide on the surface of the arsenic sulphide particles takes place up to 100 per cent. Then the new part of the curve begins which indicates no fresh formation of hydrogen ions. At higher dilutions, the hydrogen ion concentration changes very little with dilution on account of progressive hydrolysis of arsenic sulphide particles. Colloidal solutions of tungstic acid also gave peculiar conductivity and hydrogen ion activity curves on dilution, which were assumed to indicate a change in the degree of dispersion with dilution.

3. Pallmann's [1930] observations (which have been referred to by Mukherjee Roychoudhury, Dasgupta and Chatterjee, [1932]) also give the impression that the pH of the suspensions studied by him varies with the dilution in a manner which is different from the behaviour of true acids.

4. Salter and Morgan [1923] in their study of the effect of soil/water ratio on the pH value of acid soils found that the results can be expressed in the form of the usual adsorption isotherm. Bradfield [1924] found however that the hydrogen ion concentration of colloidal acid clay increases with the concentration of the colloid in a way characteristic of a weak acid. He therefore joins issue with the point of view of Salter and Morgan and emphasises that the clay acid behaves like an acid in true solution. Salter and Morgan support the usual physical point of view which regards the exchange acid produced by neutral salts as a result of the splitting up of the salt by the adsorption of the alkali (so called hydrolytic adsorption). An objection to the ' true acid theory ' of soil acidity consists of the fact, that equivalent amounts of different strong bases (sodium hydroxide and calcium hydroxide) are not required in electrometric titrations of acid soils. Bradfield points out that such results are usually obtained when a relatively large ratio of soil to reacting base is used and he suggests that " such conditions favour complex side reactions in which the solubilities of the resulting salts play a role." Such a

large ratio of soil to reacting base is present in the usual procedure in electrometric titrations of weak acids, when the acid is taken in the titration vessel and the alkali is added from outside. On account of the hydrolysis of the resulting solution, it is difficult to find out the end point. Using the reverse procedure equivalent quantities of both bases are required to neutralise the same amount of the clay acid and he considers that this stoichiometric equality shows that the reaction cannot be an adsorption process. The suggestion, that the non-equivalence of the two bases (when the acid is titrated by the base) is brought about by complex side reactions, is not completely free from objection, as such differences between the quantities ot two strong bases required for the neutralisation of a colloidal acid, actually exist [Pennyquick, 1930]. It is difficult to account for such differences from theoretical considerations based on solubility differences. The further observation of Pennyquick that hydroxyl ions disappear by chemical reaction with 'anhydride' acid molecules through the formation of fresh anions on the surface shows that the interaction with hydroxyl ions cannot always be attributed to the neutralisation of hydrogen ions.

5. The conception of an equilibrium between the hydrogen ions, the anions and the undissociated acid which obtains in the case of an acid in true solution is inapplicable to these colloidal systems, because the total neutralisable acid is not determined by the total amount of the acid substance present but by the amounts of the acid in a state of true solution and on the surface of the particles. The preceding observations show that the latter quantity does not change proportional to the dilution. The total quantity of acid, which enters into the equilibrium, depends on the degree of dispersion, and may also depend on the concentration of the alkali and the time of reaction. The effect of the amount of the solid phase and of the time of interaction have been already studied with reference to simple systems [Mukherjee and Sen, 1931]. The titration curves of cinnamic and isophthalic acids [Mukherjee and Sen, *loc. cit.*] show that the *apparent* strength, or dissociation constant, of the acid depends on these factors. In the case of colloidal solutions of insoluble acid substances like silicic acid or colloidal acid clay, the conditions of the equilibrium are still more complex; for, neither the colloidal acid nor its salts are in true solution as is the case with cinnamic acid. The apparent dissociation constant has no real significance. A discrepancy probably also exists [Mukherjee, 1932] between the conductivities of solutions of colloidal substances, calculated from the activities of ions, and those directly observed. The theoretical considerations advanced by Mukherjee [1921, 1922] offers a basis for the correlation of the observations. Colloidal particles of acidic substances owe their negative charge to the primarily adsorbed anions on the surface. The conception of the primary adsorption is distinct from the usual

conceptions of the adsorption of ions. Adsorption has been and is often attributed to changes in interfacial energy without any indication as to the location of the adsorbed ions within the interface. A distinction between the fixation of an ion on the surface of the solid and its presence in the liquid side of the interface is however necessary. The stability of the primarily adsorbed ions results from the 'building in', so to say, of these ions in the lattice structure of the solid colloidal particle or of its solid surface layers only and is associated with the magnitude of the potential energy. Subsequent adsorption of oppositely charged ions on the solid surface is the result of electrostatic forces or of chemical affinity. Even when the chemical affinity is weak the electrostatic adsorption is alone sufficient under suitable conditions for the fixation of ions of opposite sign on the charged surface. This secondary adsorption resulting from electrostatic forces has been termed 'electrical adsorption' and consists in the union of primarily adsorbed ions with the electrically adsorbed ones. If the latter are poly-valent the possibility arises of forming complexes which carry a charge opposite in sign to that of the primarily absorbed ions. In systems where the chemical affinity referred to above is negligible such a reversal of the charge of the surface can be attributed purely to electrical adsorption. If we take the case of a colloidal acid solution of silicic acid, the distribution of the ions in the mobile sheet of the charged colloidal particles will remain unaffected by the number of such colloidal particles in unit volume so long as collisions between these particles are small and, in consequence, do not affect the distribution of the ions between the mobile and the primarily adsorbed layers. Such a particle may contain hydrogen ions both in the primarily adsorbed layer and moving freely in the mobile sheet, and behave as a strong acid for very low dilutions, though the total acidity will be greater than the free acidity. It may thus have the character of an undissociated acid as also of a strong completely dissociated acid. The possibility of the adsorption of hydroxyl ions by chemical affinity is an important factor in determining the total neutralising capacity of the acidic substances. Thus alkali may disappear through the fixation by adsorption of the hydroxyl ions on the surface, *e.g.*, through combination with silicon dioxide molecules, and not only by a neutralisation of the hydrogen ions on the surface.

7. The preceding discussion shows that colloidal acids do not behave as acids in true solution and their special features consist in the following: The total acid entering into equilibrium is not determined by the total quantity of the acidic substance in the system. The time of interaction, the degree of dispersion, the total amount of the substance present in the system, and the adsorption of hydroxyl ions (as distinct from the neutralisation of hydroxyl ions by the hydrogen ions associated with the colloid) or of other anions in a smaller extent, are possible factors which determine the total

quantity of the acid entering into the reaction. An acid, *e.g.*, silicic acid, known to be very weak in a state of true solution—on the basis of the usual principles of electro-chemistry—behaves in some respects as an exceptionally strong acid when present as a colloidal solution. The corresponding *solid* acids or *solid* salts have no unequivocal existence and discussions of dissociation constants or equilibrium conditions in terms of such solubilities lack in reality [Mukherjee, 1929]. According to classical electrochemistry the basicity of a colloidal acid (assuming the particles to be of the same size and shape) should be given by the total number of neutralisable hydrogen ions associated with it. It has been stated above that this number does not give a true indication of the total neutralisable acid in the system nor its variation with the dilution of the sol. The number of the primarily adsorbable or actually adsorbed anions with which the hydrogen ions are associated in a free or adsorbed state, depend on the extent of the interface and possibly also on the pH (*e.g.*, on the alkaline side). This indefiniteness of the total neutralisable acid need not necessarily be the common characteristic of all such systems and indeed even of the same substance. Thus the particles of a *very finely dispersed* acidic substance may react very quickly and the total acid estimated experimentally may be identical with the total amount of the substance. Also systems may be met with in which the surface of the particles is fully saturated by the primarily adsorbed anions and the degree of dispersion does not change with the dilution. There however remains an essential difference. The basicity as defined above probably has not the same significance as in the case of polybasic acids in true solution. In the light of the theoretical picture discussed in the preceding paragraph the nature and basicity of the primarily adsorbed anions should be the relevant factors in determining the equilibrium conditions. It appears to be erroneous to consider the stages of dissociation of the total number of hydrogen ions without reference to the character of the primarily adsorbed ions. So far the acidic substance has been considered to be in a state of uniformly dispersed colloidal solution. But the state of aggregation or the structure of the gel possibly enters as additional factors in determining the bebaviour of the acid constituents of soil. Besides the association of the sesquioxides, either in a free state or in combination, with soil acids raises other interesting considerations. Possibly only a small amount of the aluminium or ferric ions, associated with the soil occurs in a state of free solution and the manner in which these ions in soil enter into reactions involving hydroxyl or hydrogen ions, is of considerable interest. The strong electrical adsorption of these trivalent cations by primarily adsorbed anions, which often leads to a reversal of the charge, is well known [Mukherjee and coworkers, 1926 ; Ghosh, 1926]. Surface complexes of various types may result profoundly affecting the interaction of the system with acids and alkalis. It would thus appear

that colloidal acid systems would show a manifold variation in their behaviour. A comprehensive theoretical treatment on quantitative lines awaits fuller experimental material dealing with various systems, of which the acid constituents of soil form a very interesting group.

Titration curves of silicic acid sols and aluminium oxide sols.

An illustration of such variations is afforded by the existing data relating to silicic acid sols. While Pauli and Valko [*loc. cit.*] find that their sols are completely dissociated, Rabinowitsch and Laskin [*loc. cit.*] find that these sols are partially dissociated. It has been shown previously [Mukherjee *et al*, 1932] that the total acid is greater than the free acid and this is approximately in agreement with Rabinowitsch and Laskin. But the variation of the hydrogen ion activity, with the concentration of the sol, is more in conformity with the conclusions of Pauli and Valko. The difficulties of the relevant measurements and the great influence exerted by traces of electrolytic impurities have been referred to in previous papers. In some of the potentiometric titration curves more than one break was observed simulating the curves of a polybasic acid with dissociation constants which vary considerably with the stage of dissociation. Similar breaks have been recorded in the literature on the soil acids. It appears that the breaks in the curves observed by us are possibly to be attributed to the peculiarities of the kinetics of the interaction between the colloid and the added alkali. (It is necessary to carry the titrations to much higher pH values than we have done so far for further information). The titration curves observed by us differ in some respects from those of Rabinowitsch and Laskin [*loc. cit.*] as also of Pauli and Valko [*loc. cit.*]. It was thought desirable to continue the work on the silicic acid sols and also to ascertain the changes brought about by 'ageing', which results in the formation of visible floculi. The sol used in this investigation contained a considerable amount of sodium chloride. This has the advantage that the hydrogen ions associated with the colloid may be exchanged and of showing any effect such an exchange may have on the titration curves. The silicic acid sols used in this and the previous paper differ in this respect from the sols used by the authors referred to above.

2. The results of titration of aluminium oxide sols, containing traces of hydrochloric acid, with alkali have been given by Mukherjee *et al* [1932]. The extent of combination of aluminium oxide in a colloidal state with hydrochloric acid to form adsorbed aluminium ions has been investigated. For this purpose it is necessary that the sol is as free as possible from hydrochloric acid (free or combined) and the pH is near the neutral point. Under such conditions the concentration of the aluminium ions in a state of true solution should be very low. Such investi-

gations would give an idea of the extent to which the range of pH values in which the adsorbed aluminium ions serve as reservoirs of potential acidity. It is hoped to deal more fully with several aspects of such reactions in later papers.

3. In a previous contribution [Mukherjee, *et al*, 1932], a method for simultaneous potentiometric and conductometric titrations has been described. The present paper records : (*a*) results of such simultaneous titrations with a sample of silicic acid sol at different dilutions, and (*b*) results of potentiometric titrations with two samples of aluminium hydroxide sols of very low specific conductivities. As suggested in previous parts, traces of carbon dioxide or oxygen cause considerable error, and for the purposes of a quantitative interpretation of the results these sources of error should be avoided. In the present series of measurements great care was taken to exclude carbon dioxide. Nevertheless some of the titration curves were not very regular. Perhaps traces of carbon dioxide could not be entirely avoided. It was also felt in the course of the investigation that the passage of a stream of hydrogen gas may not ensure an efficient mixture of alkali and acid. Further improvements eliminating these troubles have been made with the collaboration of Mr. S. C. Ganguly, M.Sc. The results given below were obtained with the arrangements described by Mukherjee *et al* [1932], and the results obtained by the recent improvements will be communicated in a later paper.

Experimental.

Preparation of silicic acid sol:—The silicic acid sol was prepared by adding 525 c.c. of sodium silicate solution of specific gravity 1·16 to 910 c.c. of hydrochloric acid solution containing 210 c.c. of concentrated hydrochloric acid. The mixture was dialysed against repeated changes of cold distilled water until a weakly opalescent sol was obtained. It was stocked in an atmosphere of hydrogen [Mukherjee, *et al*, 1932.]

Preparation of aluminium hydroxide sols (K *and* M) :—Aluminium hydroxide precipitated by adding a litre of 0·1*N* ammonium hydroxide to a litre of 0·1*N* aluminium chloride solution was washed repeatedly with conductivity water by the help of a centrifuge until a partial peptisation took place. A portion of the residual precipitate, when suspended in water, gave a fairly stable suspension.

(*i*) To one part of the suspension two litres of conductivity water were added and a sol was obtained (*K*).

(*ii*) On adding 2 litres of conductivity water containing 1 c.c. of ·005*N* hydrochloric acid to another portion, sol *M* was formed.

The sols were stocked in an atmosphere of hydrogen [Mukherjee, *et al*, 1932].

Measurement of hydrogenion concentration.—The hydrogen ion concentrations of the sols were measured at 35° ± ·1°C. by means of the hydrogen electrode or by the quinhydrone electrode against normal calomel electrodes. A K-type Leeds Northrup potentiometer was used. The e. m. f. remained remarkably constant during all the measurements given in the following tables.

Preparation of hydrogen.—Pure electrolytic hydrogen was prepared by the method already described by Mukherjee, *et al* [1932].

Simultaneous potentiometric and conductometric titrations of the sols.—The apparatus described for this purpose by Mukherjee *et al* [1932] was used. The conductivity measurements were taken at 35° ± ·1°C. by means of a Leeds Northrup conductivity bridge and Curtis coil resistances.

Determination of chlorine ion concentration.—The chlorine ion concentrations were measured by Hg—HgCl electrodes. The accuracy of the electrodes was tested before use.

The estimation of silicon dioxide in silicic acid sol.—A measured volume of the sol was evaporated to dryness in a platinum basin on a water-bath three times with concentrated hydrochloric acid, digested with acid and water, filtered, and the filtrate collected. The residue with the filter paper was dried and ignited in a platinum crucible and the silicon dioxide weighed.

Results and discussion.

(*I*) *Simultaneous potentiometric and conductometric titrations of silicic acid sol* (A) *and its dilutions.**—Between two successive readings there was a constant time interval of 30 minutes.** 50 c.c. of sol were taken in each titration.

* At the time of these titrations partial coagulation of the sol took place.
** The strength of the baryta solution (·059*N*) remained satisfactorily constant during the titrations.

TABLE I.

Silicic acid sol (A)

Concentration of silica = ·4276 grm. molecule per litre.

(Figs. 1 to 5, Curves I to X)

The following calculated data were corrected of change of volume.

A			A/2			A/6			A/11			A/21		
Baryta added c.c.	pH (Curve I)	Sp. cond. × 10^5 (Curve II)	Baryta added c.c.	pH (Curve III)	Sp. cond. × 10^5 (Curve IV)	Baryta added c. c.	pH (Curve V)	Sp. cond. × 10^6 (Curve VI)	Baryta added c. c.	pH (Curve VII)	Sp. cond. × 10^6 (Curve VIII)	Baryta added c.c.	pH (Curve IX)	Sp. cond. × 10^6 (Curve X)
0	1·47	486	0	1·44	280	0	2·47	603	0	2·08	382	0	2·42	295
1	1·59	394 (?)	1	1·58	253	·5	2·72	592	·2	2·42	330	·1	2·60	281 (?)
2	1·73	480	2	1·73	230	1·0	3·22	481	·4	3·00	312	·2	2·77	298 (?)
3	1·88	443	3	1·73	207	1·5	6·80	471	·6	4·02	290	·3	3·05	285
4	1·92	426	4	2·04	191	2·0	7·63	459	·8	6·32	290	·4	3·00(?)	292 (?)
5	2·19	468 (?)	5	2·48	173	2·5	7·69	457	1·0	7·27	283	·5	3·47	271
6	2·33	296	6	4·81	169	3·0	7·98	488	1·2	7·50	296	·6	5·70	245
7	2·70	442	7	..	..	3·5	8·04	522	1·4	7·63	316	·7	7·01	259
8	3·21	532	8	..	..	4·0	8·09	550	1·6	7·68	332	·8	7·22	283
9	3·33	542	9	5·30	166	4·5	8·22	575	1·8	7·86	338	·9	7·63	305
10	3·99	575	10	5·74	169 (?)	5·0	8·29	581	2·0	7·91	343	1·0	7·67	309 (?)
11	5·20	612	11	5·98	161	..	..	..	..	..	..	1·1	7·85	366
12	5·51	622	12	6·07	163	..	..	..	..	..	..	..	..	..
13	5·72	649	..	..	..	..	..	..	..	..	..	..	..	..

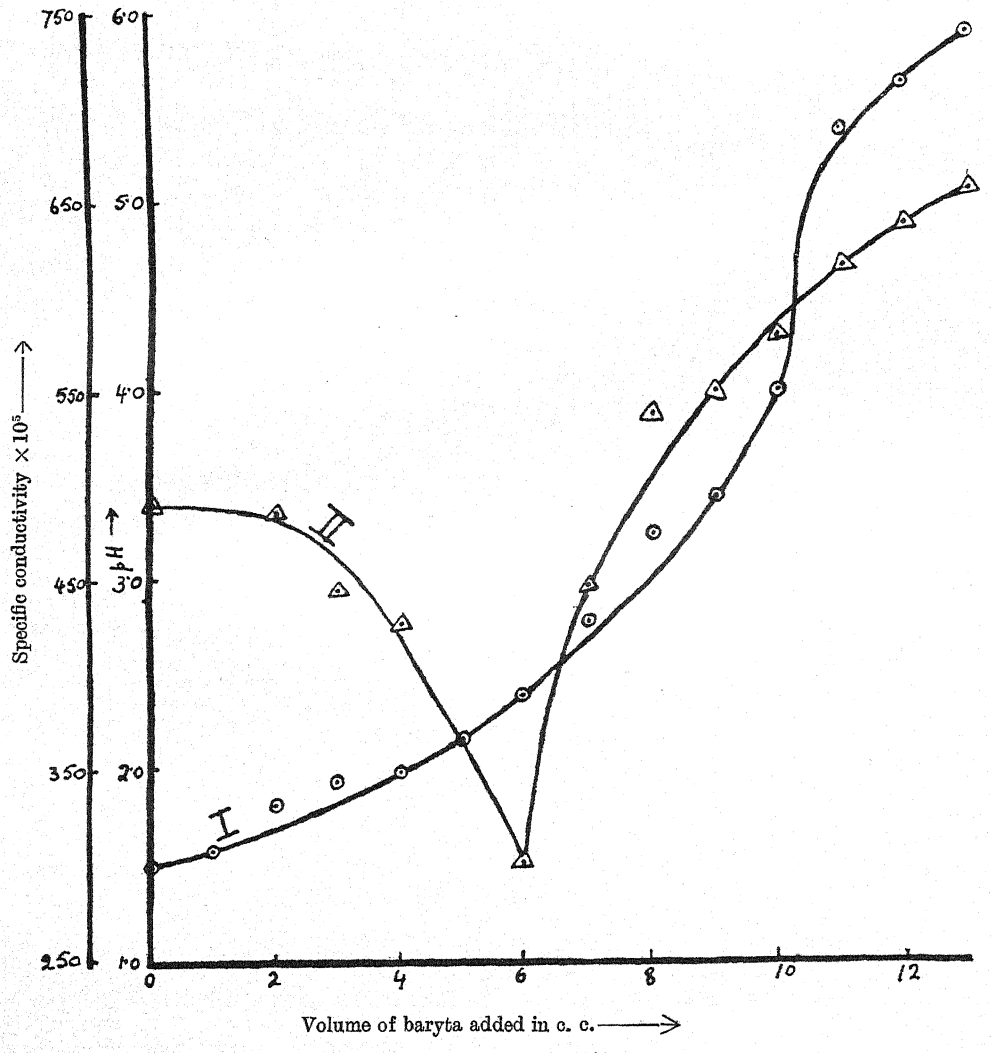


Fig. 1. Silicic acid sol (A).

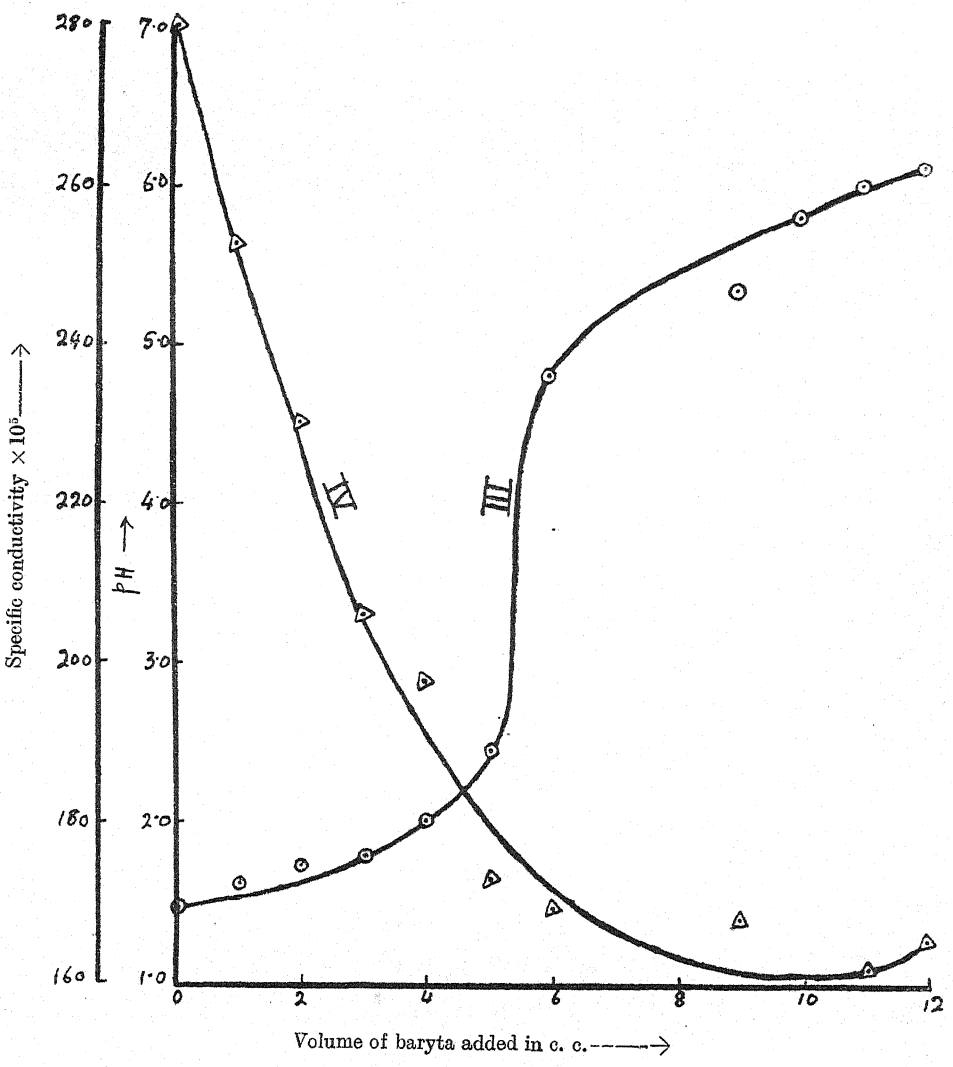


Fig. 2. Silicic acid sol (A/2).

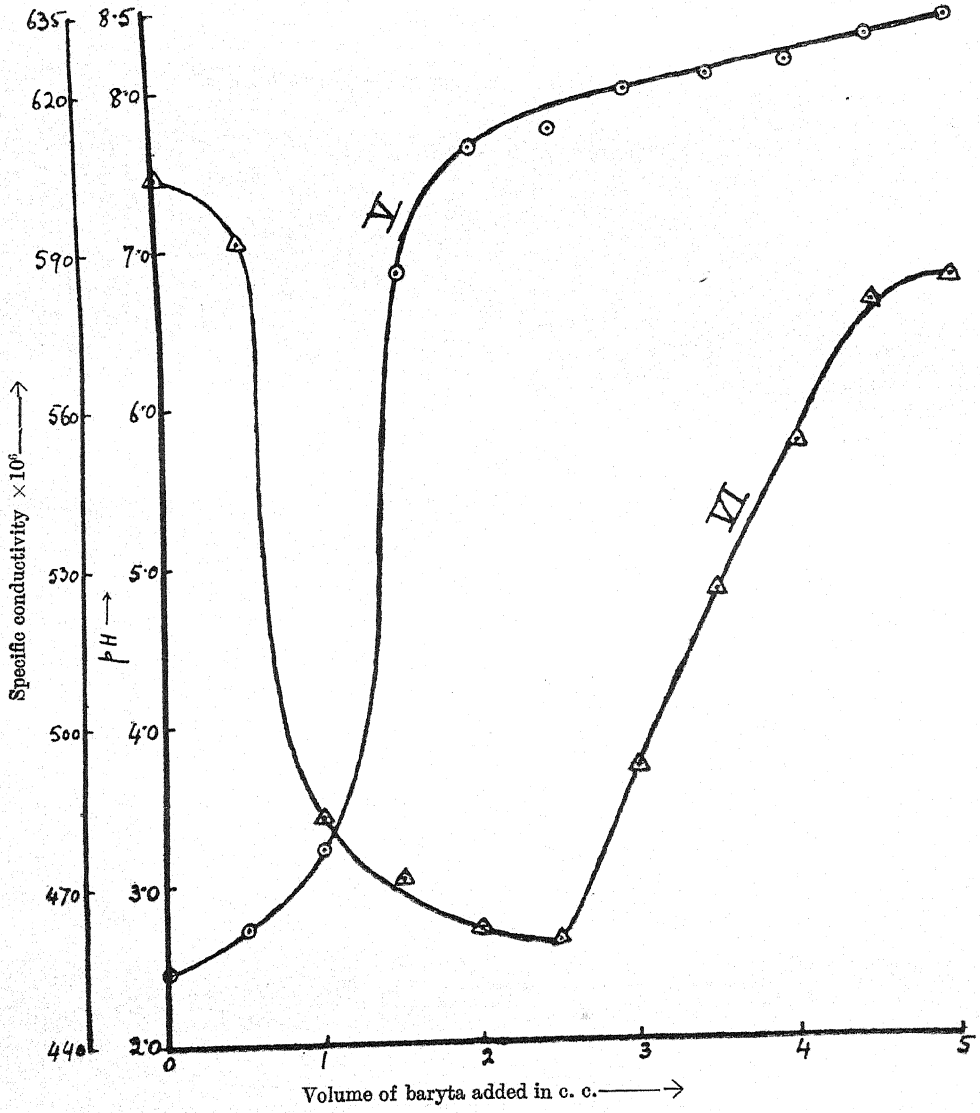


Fig. 3. Silicic acid sol (A/6).

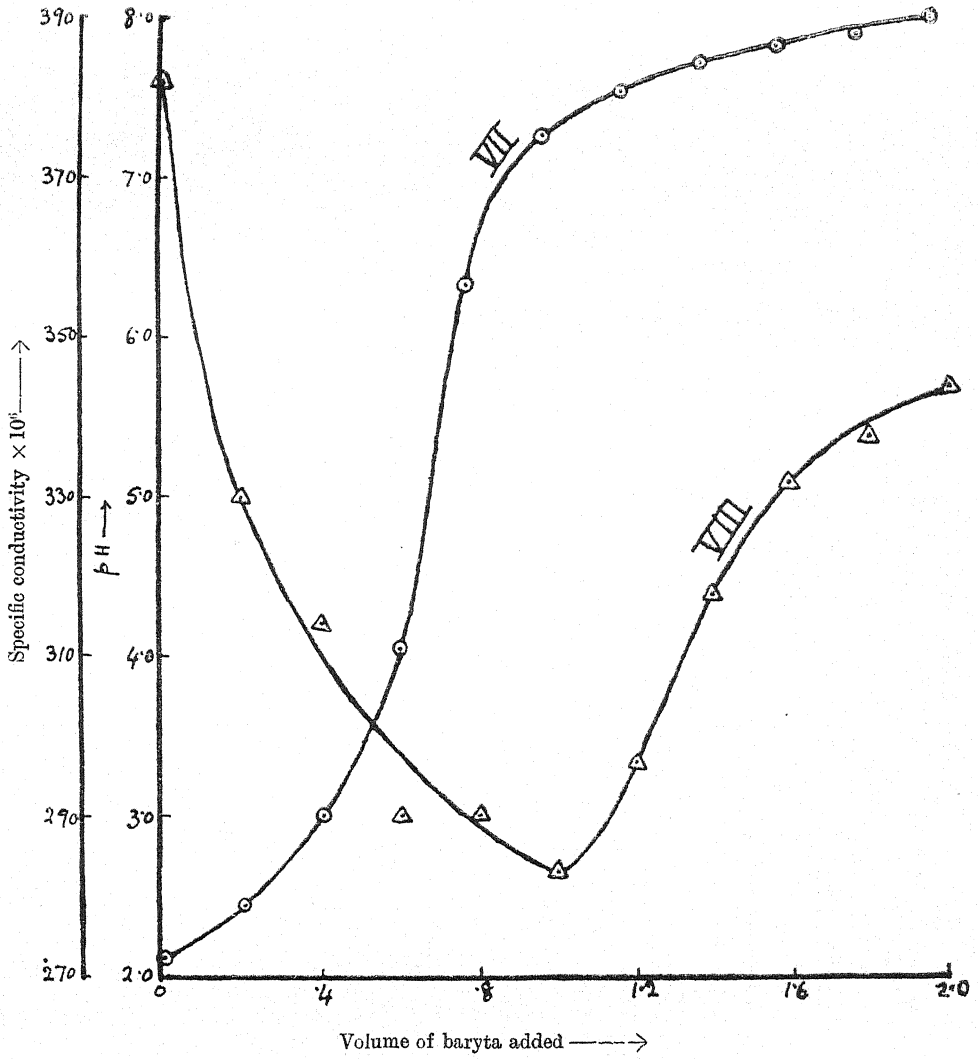


Fig. 4. Silicic acid sol (A/11).

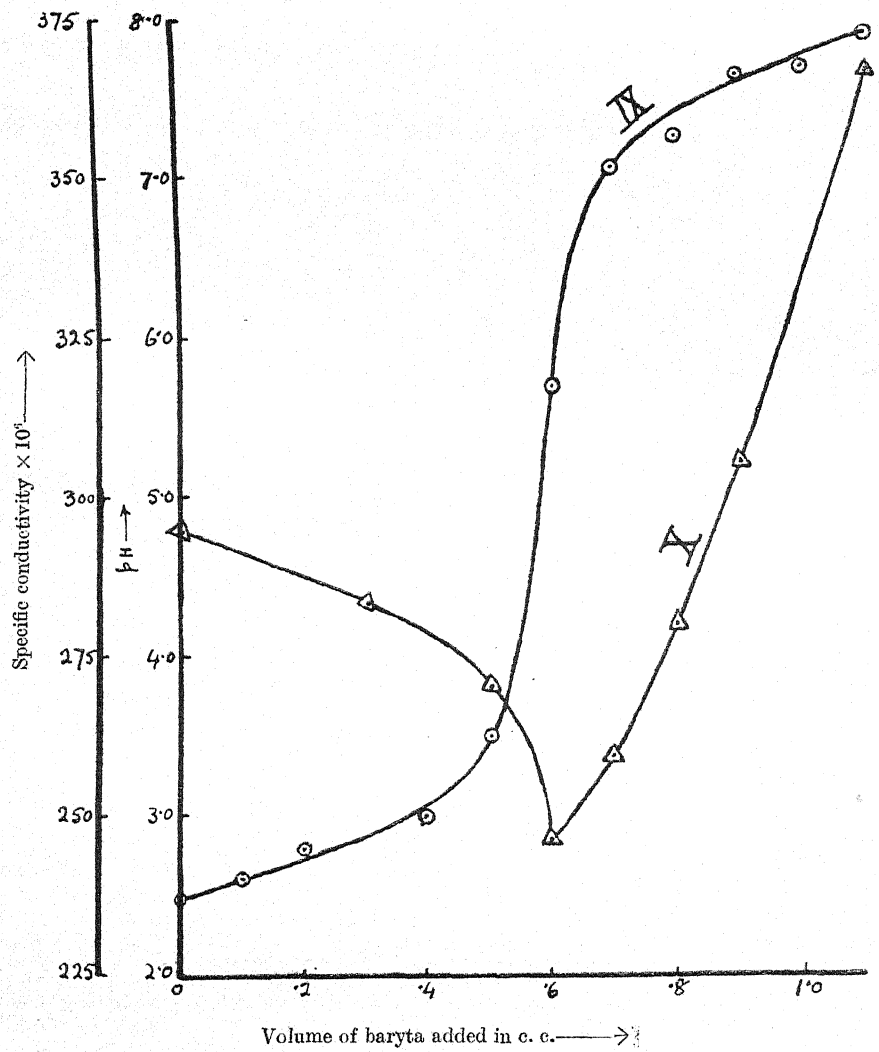


Fig. 5. Silicic acid sol (A 21).

(*II*) The total neutralisable acid as obtained from the conductometric and e. m. f. titration curves have been given in grm. ions of hydrogen per litre in Table II.

TABLE II.

Dilution	Free acidity before coagulation (Curve XI*, Fig. 6)	Free Acidity when signs of coagulation were observed	Sp. cond. $\times 10^5$ before coagulation	Sp. cond. $\times 10^5$ after signs of coagulation were observed	Total Acidity in grm. hydrogen ions per litre from	
					Conductometric titration	Potentiometric titration
A	·0132	·034	· 684	486	·00708	·0124
A/2	·0059	·036	376	280	†	·00649
A/6	·0019	·0034	130	60·3	·00283	·00153
A/11	·00084	·0083	75	38·2	·00109	·00087
A/21	..	·0038	..	29·5	·00071	·00070

* The free acidity values were obtained by hydrogen electrode (Table III).

† No definite minimum point in the conductometric titration curve was obtained.

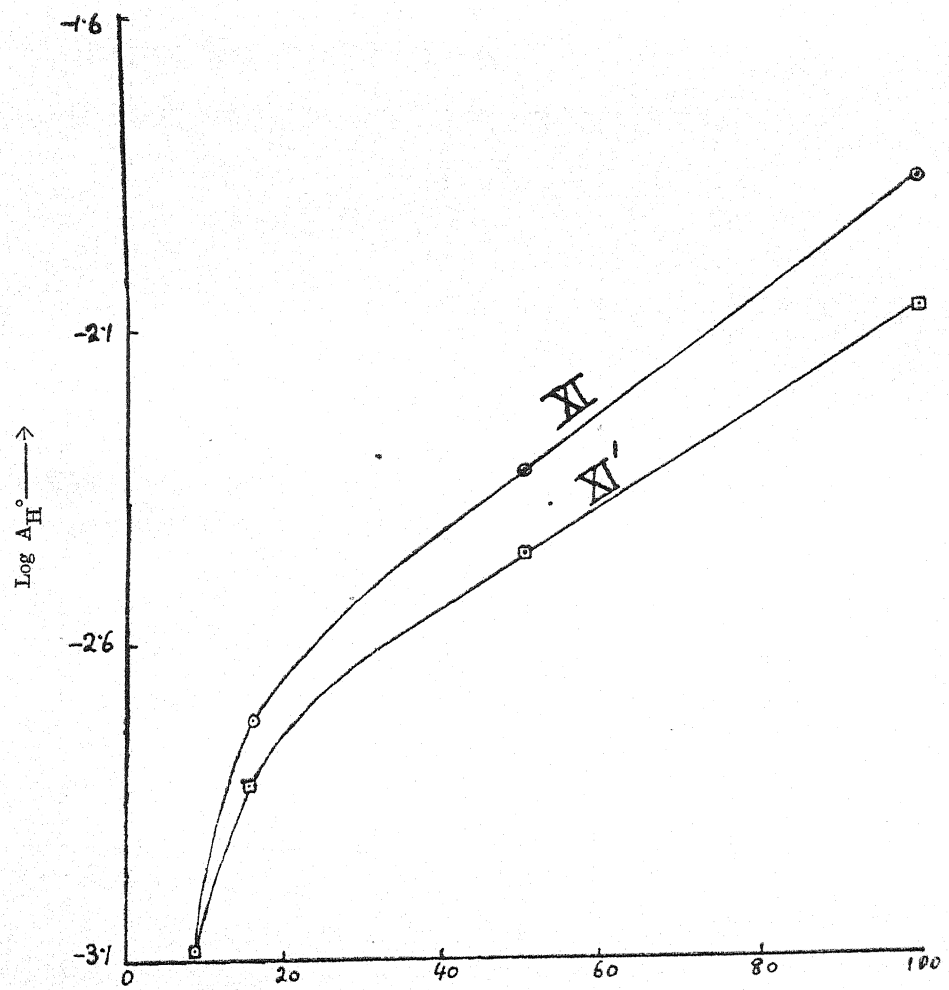

-1·6
-2·1
-2·6
-3·1
Log A_H° ——>
XI
XI'
0
20
40
60
80
100
Relative concentration.

Fig. 6.

1. Table III below represents the agreement between the pH values of the silicic acid sol (A) obtained by both the hydrogen and the quinhydrone electrodes.

TABLE III.

Dilution :		
A	1·879	(H_2 electrode)
	1·96	(Quinhydrone electrode)
A/2	2·23	(H_2 electrode)
	2·33	(Quinhydrone electrode)
A/6	2·71	(H_2 electrode)
	2·42	(Quinhydrone electrode)
A/11	3·076	(H_2 electrode)
	3·051	(Quinhydrone electrode)

Curve XI in Fig. 6 gives the variations in log A_H° of the sol A with concentrations. Curve XI′ in the same figure shows the variation in the log A_H° of four corresponding hydrochloric acid solutions, the least concentrated of which is such that it has the same A_H° as sol A/11. A corresponding series of curves has been given in Fig. 7, which have been obtained from the data on silicic acid sol given in a previous contribution [Mukherjee, *et al*, 1932]. Curves XII and XII′ have been obtained by the same manner of calculations as used in and correspond to curves XI and XI′.

2. The sol contains free electrolytes, hydrochloric acid and sodium chloride. The concentration of these electrolytes increases proportionately with that of the sol. Moreover, it is well-known that the presence of neutral salts like sodium chloride solution in contact with silica causes increased acidity [Mukherjee and coworkers, 1926]. The ionic strength of these solutions is greater than that for hydrochloric acid solutions having the same pH. The activity coefficients should therefore be smaller and also decrease more rapidly with the concentration. Mukherjee, *et al* [1932] have discussed the possibility that the e. m. f. of the hydrogen electrode may be in error. The agreement between the quinhydrone and hydrogen electrodes is definitely against it. Rabinowitsch and Laskin [1928] have also found that both these electrodes give results in mutual agreement.

3. A rather good agreement is noticed between the total acidity after partial flocculation and the free acidity measured before flocculation (Table II). But the free acidity appears to increase considerably in each case on ageing and shows irregular variations whereas the specific conductivity of the sol decreases. The potentiometric titration curves, given later, have a normal form and this constitutes a difficulty in attributing the surprisingly large increase, on ageing, of the free hydrogen ion activities to the unreliability of the hydrogen electrode. The

decrease in the conductivity is also in contrast to the agreement between the free and total acidities mentioned above. The changes brought about by flocculation therefore require further investigation. The manner of variation of the hydrogen ion concentration with the dilution of the sol is typical of a completely dissociated acid and in agreement with previous results [Mukherjee, *et al*, 1932]. In consideration of the greater ionic strength and the polyvalent character of the silicic acid particles the A_H° curves indicate that the sol is a stronger acid in this respect than hydrochloric acid.

(*III*) *Electrometric titration curves of silicic acid sols* (*Table I, Figs. 1-5, Curves I-X*).—Greater precautions have been taken in the present work for excluding traces of air from the titrating vessel. The runs of the titration curves are more regular than they were previously [Mukherjee, *et al*, 1932]. Although the difficulties previously mentioned have been eradicated to a large extent it seems that a more efficient stirring of the sol than that secured by bubbling hydrogen gas should be ensured. Attempts were made to measure simultaneously the gradual variations of chlorine ion concentrations in the sol by Ag-AgCl electrode. It was found at the end of the titrations that these electrodes were unreliable. The causes of the poisoning are under investigation. The chlorine ion data have therefore been omitted from Table I.

2. Table II shows that except in the case of the pure sol the value of total acidity calculated from the conductometric titration curve is greater than that from the potentiometric titration curve. We find that the total acidity values given by the conductometric titration curve, at all dilutions except with the pure sol, are greater than the free acidity values, measured before coagulation, while only with sol A/21 the total acidity values given by potentiometric and conductometric titrations agree. The extremely low value of total acidity of the pure sol by conductometric titrations is indeed difficult to explain. The results evidently show that disagreements are more prominent with concentrated than with diluted sols. There is one factor which might have caused some disturbance in conductometric titrations, specially with concentrated sols. The distance between the two electrodes in the titration vessel was rather small and with comparatively concentrated sols it was likely that some of the silicic acid might have set into flocculi in the space between them. The sol as a whole was fluid and the form of the conductivity curves indicates that this disturbance may be excluded. In contrast to the findings of Rabinowitsch and Laskin [*loc. cit.*], the shapes of the conductometric titration curves do not resemble those of strong acids. On the contrary, these curves represent more the conductometric titration curves of moderately strong or weak acids. Curve IV forms an exception. Barium silicate is known to be

sparingly soluble. Considering the form of the A_H° curve and the agreement between the free and total acidities mentioned previously the conductivity curves should have only steep branches. The curves therefore have an unexpected form and indicate that the neutralisation of hydrogen ions by the alkali and the formation of insoluble barium silicate or the electrical adsorption of barium ions are not the only reactions taking place in the system. Both branches of the curves indicate a reaction between the interface and the hydroxyl ions, whose kinetics are of a complicated nature. The descending portions show that processes resembling hydrolysis of salts are taking place. Possibly barium ions liberate hydrogen ions from the interface. These hydrogen ions do not appear to have been in an undissociated state in view of the agreement between the free and total acidities. A difference in the conductivity coefficients of the hydrogen ions in the double layer may be imagined but such explanations would be highly speculative in the absence of more complete data.

3. The potentiometric titration curves, when closely examined, also show a departure from the normal form for a strong acid. The curve for sol A does not show the usual steep rise following a flatter portion. The form of the curve of course depends to some extent on the scale used for plotting it but the values of the tangent $\delta E/\delta \chi$, where δE represents the change in the e. m. f. produced by $\delta \chi$ gram equivalents of the alkali, which should be used, justify this statement and give an idea of the extent to which the curves differ from that of a strong acid. It is remarkable that as the dilution proceeds the curves for both sets of titrations, conductometric and potentiometric, approach the forms characteristic of strong acids. It is also to be noted that the total acidity given by both titrations agree for sol A/21. The potentiometric titration curve, which undergoes a sharp change near the neutral point, is not likely from its nature to show as marked a departure in form as the conductivity curves. It thus appears that the departure from the normal form and the resemblance with the form of weak acids is real and some sort of interaction involving the interface takes place.

4. Regarding the possible sources of error a chance of the access of ammonia or carbon dioxide into the titration vessel is to be excluded in view of the special arrangement used and of the observed steady e. m. f. and conductivities. The irregular distribution of the points in some of the titration curves referred to before might result from the absence of a more vigorous stirring. The presence of a reaction involving an interface would aggravate the difficulties in the way of ensuring a rapid attainment of the equilibrium. The addition of the baryta solution was not uniform in one respect for the different dilutions in the sol. It was added in amounts of one c.c. to the sols A and A/2, of 0·5 c.c. to A/6, of 0·2 c.c. to A/11 and

of 0·1 c.c. to A/21. The difference arose out of the diminution of the total acid on dilution. Although the interval between successive additions was constant in all cases the rate of reaction might vary with the pH. Finally unequal quantities of the silica might have been transferred with equal volumes of the sol on account of the non-homogeneous dispersion after partial flocculation. The agreement between the free and total acidities show that the error from this cause cannot be considerable.

5. The results taken as a whole agree with the conclusion stated at the beginning of this paper that silicic acid sols prepared in the manner given here seems to have the character of a strong acid in some respects and of a weak acid in others. Partial flocculation does not appear to change the total acidity calculated from the potentiometric titration curves. The sols used by Pauli and Valko were electrodialysed and their sols as also those of Rabinowitsch and Laskin were free from added electrolytes. The pH of the sol A and its dilutions are considerably lower and they also contain a larger amount of silicic acid and of free sodium chloride.

(IV) Potentiometric titrations of aluminium hydroxide sols.

TABLE IV.

Aluminium hydroxide sol (K).

Interval between consecutive additions of acid = 30 minutes.
Concentration of HCl = ·001 *N*; 50 c.c. of sol was taken.
(Fig. 7, Curves XIII, XIV, XV.)

Volume of acid added (c.c.)	E. M. F. (in volts)			pH		
	I	II	III	I (Curve XIII)	II (Curve XIV)	III (Curve XV)
0·0	·6655	·6627	·6624	6·31	6·25	6·24
0·2	·6636	·6647	·6539	6·27	6·28	6·09(?)
0·4	·6642	·6612	·6673	6·29	6·23	6·32
0·6	·6543	·6617	·6685	6·12	6·23	6·34
0·8	·6225	...	·6579	5·60	...	6·15(?)
0·9	...	·6320	...	...	5·74	...
1·0	·5954	...	·6697	5·16	...	6·35
1·1	...	·6105	...	...	5·40	...
1·2	...	...	·6432	...	...	5·89
1·3	...	·5915	...	...	5·08	...
1·5	...	·5813	...	...	4·92	...
1·7	...	·5770	...	...	4·84	...

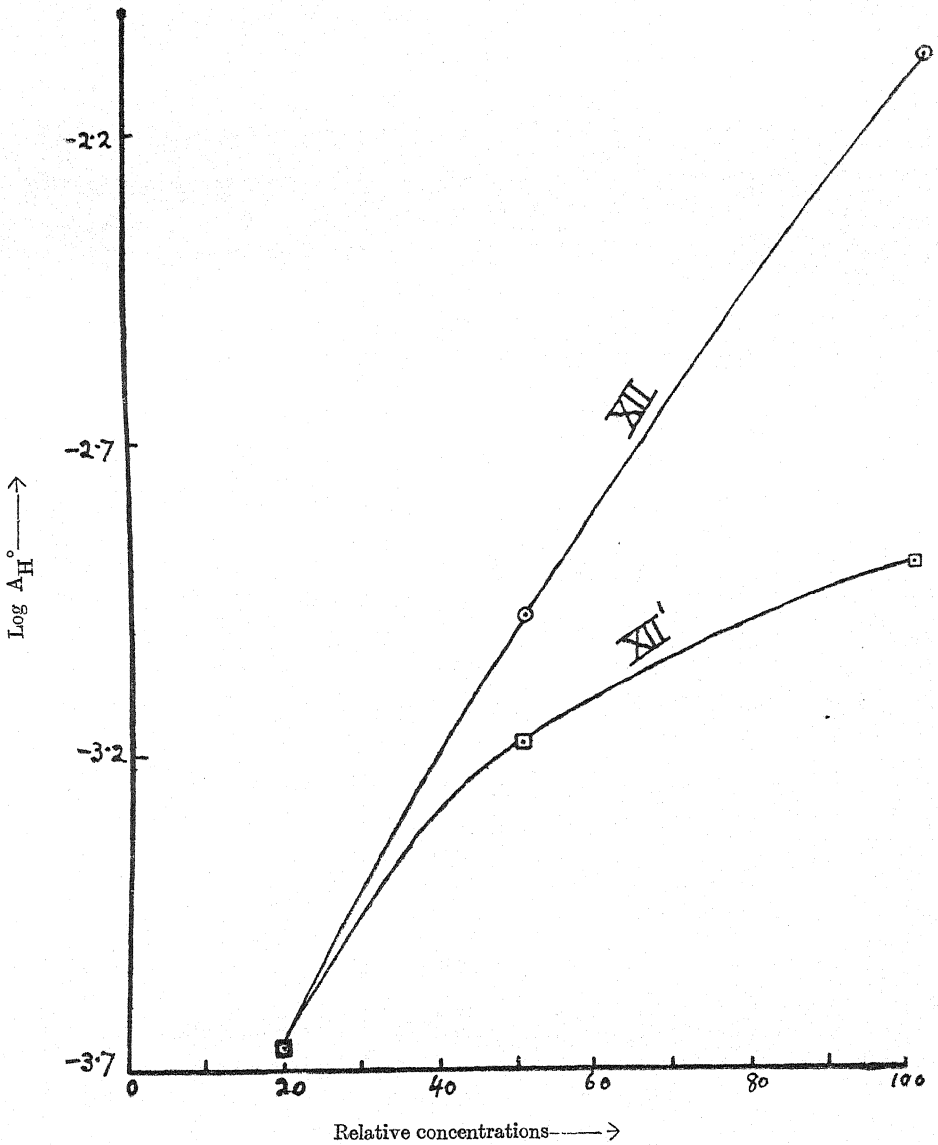

-2·2
-2·7
-3·2
-3·7
Log A_H° ——→
0
20
40
60
80
100
XII
XII'
Relative concentrations ——→

Fig. 7.

TABLE V.

Aluminium hydroxide sol (M).

Interval between consecutive additions of acid =30 min.

Concentration of HCl = ·001 *N*; 50 c.c. of sol was taken.

(Fig. 8, Curve XVI.)

Volume of acid added (c.c.)	E. M. F. in volts	pH (Curve XVI)
0	·66475	6·27
0·2	·6648	6·27
0·4	·6617	6·22
0·7	·64005	5·86
0·9	·6214	5·56
1·1	·6010	5·23
1·3	·5957	5·14
1·55	·5643	4·63
1·70	·5585	4·53
1·90	·5229	3·95

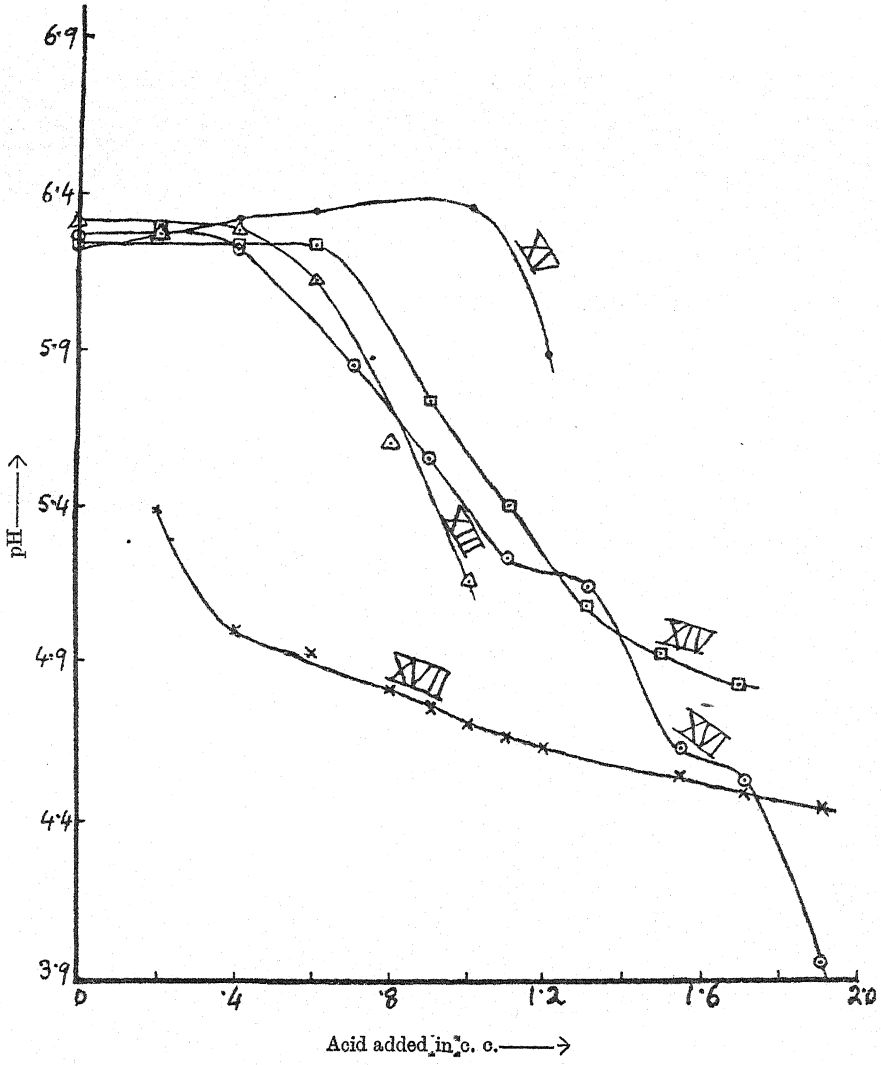

6·9
6·4
5·9
5·4
4·9
4·4
3·9
pH
0
·4
·8
1·2
1·6
2·0
Acid added in c. c.
XI
XIII
XIV
XVI
XVII

Fig. 8.

The reproducibility of the titration curves of aluminium hydroxide sol is not so satisfactory being in contrast with the reproducibility observed previously [Mukherjee, *et al*, 1932]. Perhaps a greater time for reaching an equilibrium of the reactions and more efficient stirring would be helpful. The main reason for this want of agreement is possibly the low specific conductivity and electrolyte content of these sols. Sol *K* has a specific conductivity of $\cdot 18 \times 10^{-4}$ and sol M $\cdot 0546 \times 10^{-5}$. In fact, considering the difficulties met with at such dilutions of acids, accurate reproducibility is difficult of attainment.

Three of the curves practically coincide with each other in their initial portions. The first additions of the acid have practically no effect on the pH which diminishes thereafter at a comparatively rapid rate. Free aluminium ions are unstable at such high pH values [Britton, 1932]. Curve XVII represents the calculated decrease of the pH of pure water on the gradual addition of hydrochloric acid in identical amounts. The sol reacts with hydrogen ions and adsorbs them at first completely and then in progressively smaller proportions. The adsorbed aluminium ions on the surface appear to be quite stable at pH 6·4. Comparing curve XVII with the others and from the data on the hydrolysis of aluminum ions it is possible to form a rough estimate of the amount of these ions on the surface. It is however necessary to ascertain the limits of reproducibility of the titration curves and also to ascertain the significance in terms of the concentration of hydrogen ions, of the observed e.m.f. of the hydrogen electrode. There appears to be some doubt as to the applicability *in toto* of the usual conceptions to these systems [Mukherjee, 1933].

Summary.

1. The titration curves of silicic acid sols show features which are of a complicated and unexpected nature when compared to those of acids in true solution. In agreement with previous observations they show that the sol appears to have simultaneously the character of a strong and of a weak acid.

2. Adsorbed aluminium ions present on the surface of colloidal particles of aluminium hydroxide sols are stable at pH 6·4.

Our thanks are due to the Imperial Council of Agricultural Research for enabling one of us (S. P. R. C.) to undertake this work as a Research Assistant and also for other facilities.

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A SCLEROTINIA-ROT OF *HIBISCUS SABDARIFFA* LINN.

BY

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(With Plates LI-LIII and one text-figure)

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INTRODUCTION.

In the winter of 1930-31 the seed crop of *patwa*, *Hibiscus sabdariffa* Linn., became very much affected by a fungous disease at Pusa. The disease had been observed in previous years but the crop at that time was not grown extensively and it was not considered serious. With the introduction of a new green-stemmed variety of *patwa*, *H. sabdariffa* var. *altissima* Wester, cultivation of this crop increased both on the Botanical area and the Pusa Farm and the appearance of the disease caused some concern.

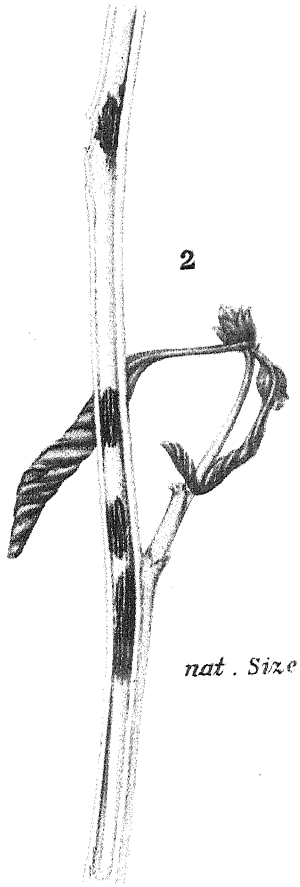
Patwa is a fibre crop and is sown soon after the first burst of monsoon rains is over, in July. It is ready for harvest as a fibre by the end of October and for the next year's seed supply a few plants are left over and this latter crop is harvested

PLATE LI.



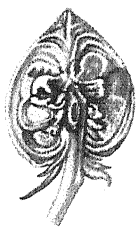
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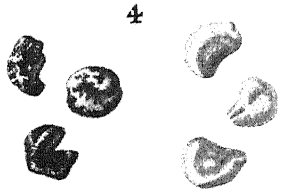
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EXPLANATION OF PLATE LI.

Fig. 1. Floral axis of *Hibiscus sabdariffa* showing the canker.

Fig. 2. Cankered stem cut open to show sclerotia.

Fig. 3. Boll from an affected plant showing sclerotia within.

Fig. 4. Sclerotia and immature seed.

in the second or third week of the following February. As the number of plants reserved for seed is rather small, particular care has to be taken of them.

Symptoms.

In 1931-32 the disease appeared on the fifth of January. From that date onwards it increased rapidly and by the end of the same month it was doing maximum damage. The crop at this time is about six months old and the flowering is almost over. Most of the leaves are shed and the only tender region is near the inflorescence.

The first evidence of the disease is a straw-brown coloured patch or canker on the main stem or the branches in the inflorescence. This patch is about six to ten feet from the ground and the stem above and below it (Plate LI, fig. 1) has a healthy appearance. The pallid colour slowly extends and finally the stem is girdled. As much as a foot or a foot and a half of the main stem may ultimately be affected. The stem at this region is less firm to the touch and it easily peels off into shreds. While the floral axis is the main part that is affected, a portion of the stem below it is also attacked and later in the season the canker extends considerably below. The Upper parts also become affected but there is no hanging down of the floral axis which remains erect.

The disease has not been found to extend to the ground level and the roots when examined have not shown injury due to this disease. Examination of the affected part with a hand lens reveals that the surface of the stem is covered by cottony strands of a mycelium which are particularly prominent in the axis of the branches with the main axis (Plate LII, fig. 6). Sometimes black irregularly round bodies can be seen on the surface of the stem and if the mycelium has spread to the base of the bolls, they can also be found in that region. Several such bolls when opened and examined have shown the presence of these black bodies (Plate LI, fig. 3). They look very much like seed and if mixed with them are hard to distinguish.

Microscopic examination of the affected parts shows that the parenchymatous tissues in the cankered region are decomposed and in the stelar region the bast fibres and the vascular bundles separate, causing shredding. Large septate hyaline strands of a fungus are found to infest the cells both intracellularly and intercellularly, and all the tissues of the stem are invaded by the fungus. The hyphae may be from 9 to 18 μ across and they are densely filled with the protoplasm. The pith may be often replaced by black structures much like those found on the surface of the stem (Plate LI, fig. 2). Sections of these structures (Plate LII, fig. 9) show that they are made of rectangular cells in the centre with rather thin walls and wide intercel-

lular spaces but at the periphery the cells are thick-walled, short and impregnated with some gelatinous material which gives these bodies their black appearance.

From a general appearance of the mycelium and the black bodies it was apparent that the fungus belonged to the genus Sclerotinia and that the black structures were the sclerotia. As this fungus is constantly associated with all the diseased plants, it looked as though it was the cause of the disease.

Fungi belonging to the genus Sclerotinia have been known to cause a stem-rot of several crop plants. In lettuce stem-rot due to *Sclerotinia sclerotiorum* (Lib.) De Bary, the disease according to Stevens and Hall [1911] starts at the base of the plant and the whole plants later show signs of wilting. Bisby [1921] and Young and Morris [1927] found in Sclerotinia wilt of *Helianthus annus* L. that the disease starts at the ground level and the cankers have a soft consistency and a water-soaked appearance. In the gray mould of castor bean, Godfrey [1923] states that the inflorescence first gets affected and that the disease later spreads to the lower leaves and stems. This disease was found by him to be due to a Sclerotinia which he called *S. ricini*. Similar is the case in Sclerotinia blossom wilt and stem-rot of *Antirrhinum majus* L. which according to Dowson [1926] starts in the inflorescence when the flowering axis droops and wilts. Further in the green rot of apricots, the cottony mould fungus, *S. sclerotiorum*, attacks, according to Smith [1931] only the blossoms and the young fruit, and the trees are not ordinarily attacked at any other part. Drooping of the floral axis is not a feature of the Hibiscus stem-rot but as in the case of Antirrhinums, castors and apricots the disease has its origin in the floral axis.

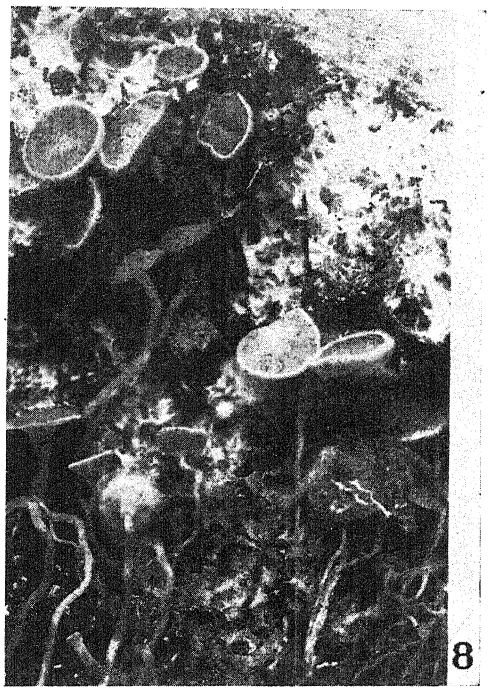
As the disease appears in the boll developing stage, those bolls that appeared before the disease had set in escape injury and yield good seed. The bolls formed later are usually shrivelled up and the seeds within are not plump and sclerotia may be found mixed up with them.

The causal organism.

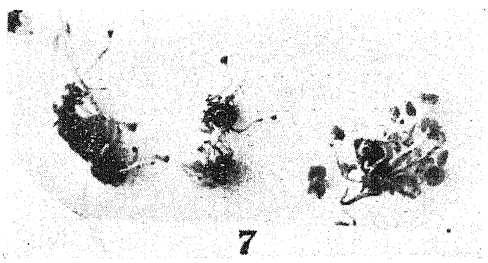
The *Sclerotinia* associated with these diseased plants is easy to isolate and three methods were used in doing so. In the first method the portion of the affected floral axis was thoroughly surface-sterilised with mercuric bichloride (1 :1000) solution and with sterilised water. This piece was placed aseptically in a moist chamber. On the third day mycelial strands were perceptible on the surface of the stem. These were carefully picked with a sterile platinum needle and transferred to a potato-dextrose agar slant. Twenty isolations were made in this way. In the second method small fragments of the affected tissue were sterilised on the surface as before, dipped in alcohol and flamed and then placed directly on potato-dextrose



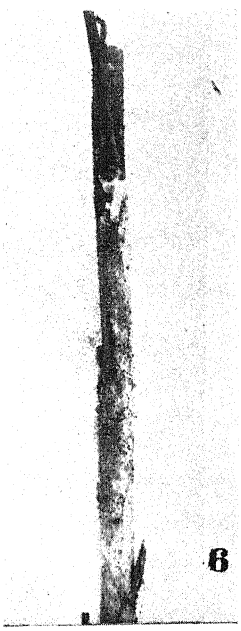
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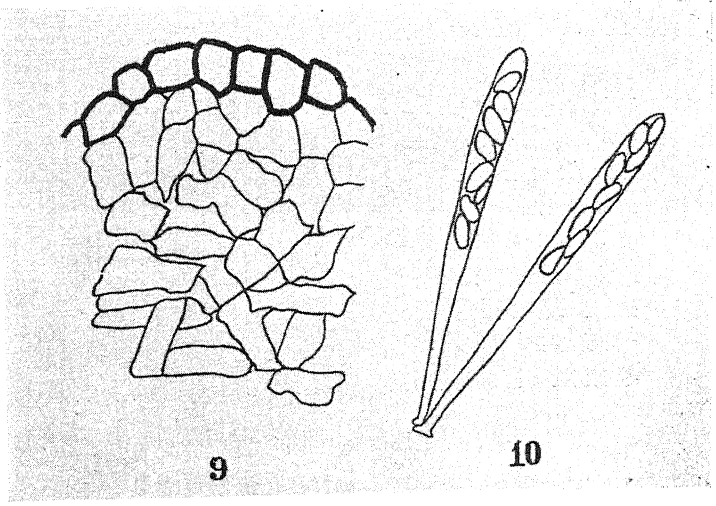
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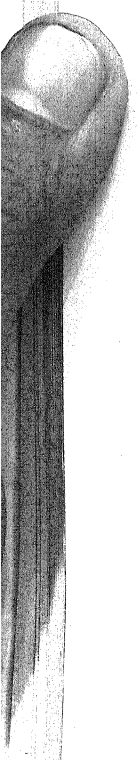


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9 10

Fig. 5. A Hibiscus plant infected a little above the soil level. The plant shows symptoms of wilting. Fig. 6. A cankered stem showing mycelial strands and cushions. Fig. 7. Apothecia produced in culture media. Fig. 8. Apothecia produced in the field. Fig. 9. A section of a sclerotium (×731). Camera lucida. Fig. 10. Asci and ascospores.



agar slants. Fungous hyphae began to appear on the fourth day and thirty-four isolations were made in this way. Finally the sclerotia were directly used for isolating the fungus. The diseased portion of the stem was surface-sterilised and split open aseptically. With sterile forceps the sclerotia in the pith were removed and transferred to forty agar slants. Fifteen of these sclerotia were dipped in alcohol and flamed before they were transferred to the agar. No conidia or other kind of spores were present on the stems or within them and single spore cultures could not therefore be made.

Ninety-four isolations were in all made, by direct transfer of the mycelium, by tissue fragment culture and by using the sclerotia. In each and every case the same fungus was obtained. All the cultures were identical showing that this one fungus was exclusively associated with the diseased Hibiscus plants.

On the potato-dextrose agar the fungus makes a good growth if the temperature of the incubator is 25° C. Within forty-eight hours copious and fluffy growth takes place. The growth is pure white and cottony and there is more of aerial than submerged growth. Sclerotia begin to form at the edges of the culture tube about the fourth day. At first they are whitish like the mycelium but later they become black bearing beads of a clear amber coloured liquid. These sclerotia looked like those found in nature on the surface of the stems and mixed within the seed but were of larger size. The former range from 1 to 8 millimeters with an average of 5·8 millimeters while the latter are from 2 to 12 millimeters with an average of 8·5 millimeters. Some exceptionally large sclerotia obtained in culture were from 8 to 20 millimeters in diameter.

The growth of the fungus on many of the media tried was good. These media were potato-dextrose agar, Quaker Oat agar, cornmeal agar, sterilised rice grains, Richards' agar, and Coons' agar. Media made with the vegetable or cereal decoctions seemed on the whole to promote better growth than the synthetic culture media. The mycelium is highly hyaline, distinctly granular and without any vacuoles. As the cultures age, the edges appear greenish black and examination shows that the colour is due to the presence of appressoria. These are flat, dendroid in shape and in mass make the old cultures appear black in colour at the edges.

Microconidia developed on potato-dextrose agar, especially in a medium to which dextrose had not been added. They appeared only when the cultures were very old and about to dry up. They are produced acrogenously on short lateral branches arising from the vegetative mycelium. This branch may end in small colourless conidia or the conidiophore may rebranch producing a crop of them. The conidiophore is short, swollen at the base and tapers to a point at the distal end where the microconidium is borne. One hundred of these microconidia were measured using

the oil immersion lens and they are from 2·5 to 5·3 μ in diameter with a mean of 4·6 μ. They are hyaline and do not show any nuclear structure within. They have not been so far observed in nature. A perusal of literature shows that endogenously produced microconidia have been observed in the genus *Sclerotinia*. None have been seen so far in this fungus. Several attempts were made to germinate these microconidia but all were unsuccessful. Until recently it was supposed that these microconidia were functionless and as lately as 1931 Smith [1931] stated 'That they have any important function, however, in the reproduction of the fungus is extremely doubtful' but work reported by Drayton [1932, 1934] with *Sclerotinia gladioli* (Massey) Drayton has shown that they act as spermatizing agency for the production of the perfect stage in that fungus.

The perfect stage.—This same fungus but isolated from *Brassica campestris* L. var. *sarson* Prain had been studied by Shaw and Ajrekar [1915] under the name *Rhizoctonia napi* West. They did not obtain any perfect stage but Joshi [1924] investigating an isolate of it from safflower, *Carthamus tinctorius* L., obtained the sclerotial processes in culture tubes. These did not produce, however, the apothicial discs. The task of producing the apothecia ot this fungus was therefore prominently kept in the forefront. In April 1932 a large number of sclerotia formed in culture media (potato-dextrose agar and sterilised paddy) and on Hibiscus plants in nature was collected and these were placed in Petri dishes containing sterilised and moistened sand or moistened sawdust. The dishes containing sclerotia from culture media and those containing sclerotia from the fields were kept separately. Five Petri dishes of each were placed in a cool compartment (10°C.) of the Frigidaire and the rest were placed in a dark corner in the laboratory. The dishes were occasionally examined and in October the sclerotia placed in the Frigidaire had put forth long stalks or processes which will be hereafter referred to as apothecial fundaments. No apothecial fundaments were observed in the Petri dishes placed under laboratory temperature conditions. Later these deteriorated as they were attacked by insects and moulds. The sclerotia in the Frigidaire were thereafter examined very frequently with jealous care but no apothecial development took place. In December as the Frigidaire needed some repairs, the Petri dishes were removed and placed against a north window. On the 9th of January 1933 when the dishes were again examined, it was found that apothecial development had taken place.

A few Roux's bottles containing potato-dextrose agar on which the fungus was growing were also placed in the Frigidaire in July 1932 and were removed at the time of repairing it. The sclerotia in these bottles had also formed the apothecial fundaments which developed into apothecial discs at the end of January. In a few other Roux's bottles that had been inoculated in October and placed at laboratory

temperatures, the fundaments appeared on the 29th of January and on the 8th of February they formed the apothecia. Some of these Roux's bottles contained potato-dextrose agar and others a cornmeal agar.

These tests indicated that temperatures of 22°C. and below were necessary for the formation of the stipes and the apothecia. At temperatures obtaining in the laboratory that were higher than this, apothecial development did not take place. The sclerotia formed on culture media and in nature readily produce apothecia. They did not seem to need a long dormant period for sclerotia obtained in culture in January produced the apothecial fundaments when shown in sterilised and moistened sawdust in February. In March they were placed against a north window but by that time the laboratory temperatures had arisen to over 22°C. and apothecia were not produced. Stevens and Hall [1911], Godfrey [1923] and Ramsey [1925] all mention the importance of light in the development of the apothecia and these experiments confirmed their observations. Two Petri dishes in which the sclerotia had formed the sclerotial processes were left in a cool incubator in the dark. These never developed into apothecia.

Identity of the fungus.— The apothecia are fawn coloured at first, broadly shallow, saucer shaped, later becoming flat or convex with a reflex margin. As they mature their colour turns to cinnamon brown to chestnut brown (Ridgway Colour Standards). The stalks or stipes are 20 to 45 millimeters long and about one millimeter at base of the disc but attenuated toward the sclerotium side. As many as five stipes have been observed on a single sclerotium and all may bear the apothecia. After they are mature, if the cover of the Petri dish is opened a small cloud of spores is discharged from these discs.

A small portion of the hymenium was mounted on the slide by teasing. The asci were in various stages of development and were intermingled with slender filiform paraphyses. The mature asci have a thickened apex where a pore is present. They are long, cylindrical but attenuated towards the base. The ascospores are hyaline, uniseriate, ellipsoidal to fusoid in shape and occupy only the upper part of the ascus. Comparing this fungus with the descriptions given by Smith [1900] and Ramsey [1925], it was manifest that it belonged to the species *Sclerotinia sclerotiorum* (Lib.) De Bary. In order to be sure that that was so, cultures of *Sclerotinia sclerotiorum*, *S. minor* Jagger and *S. intermedia* Ramsey were obtained from the United States of America. The first named culture came from Dr. H. H. Whetzel of Cornell University and bore the number S 74. The latter two were received from Dr. G. B. Ramsey of the University of Chicago under numbers 1603 and 711 respectively.

Parallel cultures of these and the Pusa isolate were run on potato-dextrose agar cornmeal agar, Quaker Oats agar and an agar medium made according to the formula

of Kotila [1929]. The colour of the mycelium of *S. minor* and S. *intermedia* was not white like that of *S. sclerotiorum* or the Pusa isolate, and the sclerotia of these latter two were also much larger in size. On considerations of habit of growth, colour of mycelium and size of the sclerotia the Hibiscus Sclerotinia showed affinities with *S. sclerotiorum* with which it was compared in detail. Apothecia of Whetzel's Sclerotinia became available in November 1933 and two hundred each of asci and ascospores of the two cultures were measured. The extreme and average measurements are recorded in Table I.

TABLE I.

Extreme and average measurements in microns of asci and ascospores of S. sclerotiorum *and the Hibiscus Sclerotinia based on 200 measurements.*

	S. sclerotiorum					
	Length			Breadth		
	Range	Mean	Coefficient of variance	Range	Mean	Coefficient of variance
Asci . .	124·0—167·4	149·0±·46	6·45per cent.	7·4—10·5	9·1±·03	7·01 per cent.
Ascospores .	8·5—16·0	13·3±·08	11·70 per cent.	4·5—8·4	6·0±·03	11·86 per cent.

	Hibiscus Sclerotinia					
	Length			Breadth		
	Range	Mean	Coefficient of variance	Range	Mean	Coefficient of variance
Asci . .	122·4—166·0	148·4±·40	5·66 per cent.	7·3—10·8	9·1±·02	5·26 per cent.
Ascospores .	8·5—15·9	13·4±·09	13·49 per cent.	4·4—8·6	5·9±·03	10·95 per cent.

It will be noted from the data recorded in Table I that the differences in mean values for length and breadth of asci and ascospores of the Hibiscus Sclerotinia and *S. sclerotiorum* are very slight. That these differences do not have any statistical

significance was calculated by the method suggested by Pearson [1911] to determine whether the two given frequency distributions can or cannot be regarded as random samples from the same general population. Mycologists have so far contented themselves in finding statistical significance by dividing the differences in mean values by the probable error of these mean differences and seeing whether odds are or are not in favour of the hypothesis. The test of agreement suggested by Pearson is more rigorous as the frequencies are compared cell by cell. The method is outlined in Table II for the determination of P in one case.

TABLE II.

Method of determining the probability that two frequency distributions are random samples of the same population. (Comparing the breadth of ascospores of S. sclerotiorum.)

a	b	$a+b$ c	$a-b$ d	$d/200$ e	e^2 f	f/c
1	0	1	1	·005	·000025	·0000250
9	6	15	3	·015	·000225	·0000150
48	57	105	—9	—·045	·002025	·0000016
57	47	105	8	·040	·001600	·0000015
40	31	71	9	·045	·002025	·0000028
34	36	70	—2	—·010	·000100	·0000014
11	19	30	—8	—·040	·001600	·0000053
1	3	4	—2	—·010	·000100	·0000250
200	200	400	0	0	..	·0000776

a=frequency of breadth of ascospores of *S. sclerotiorum.*

b=frequency of breadth of ascospores of Hibiscus Sclerotinia.

$\chi^2 = 200 \times 200 \times \cdot0000776 = 3\cdot104.$

$n = 8$ = number of frequency groups.

$P = \cdot80$ to $\cdot90$ according to Fisher's table.

Agreement therefore is very close.

The test of agreement shows therefore that there is very close relation between the two frequency distributions of the breadth of ascospores of the known and unknown species of Sclerotinia. Such values when determined for length and breadth of asci and length of ascospores of *S. sclerotiorum* and the Hibiscus

Sclerotinia, also showed close agreement, the value of P in each case being over ·80. Measurements of asci and ascospores of another *S. sclerotiorum* isolated from tobacco at Rangpur are given by Kheswalla [1934]. His asci are shorter and narrower but the ascospores are broader than the asci and ascospores of the two sclerotinias measured by the writer.

The two fungi included in this investigation are, however, same and the Sclerotinia from *Hibiscus sabdariffa* has been identified as *Sclerotinia sclerotiorum* (Lib.) De Bary. Opportunity may be taken here to point out that Massee has been considered as the authority for the generic name of this fungus. But the combination *S. sclerotiorum* was first used by De Bary in 'Vergleichende Morphologie und Biologie der Pilze', page 216, in 1884. In 1893 Schroeter in 'Kohn's Kryptogamen Flora von Schelsein' III Band, 2 halite, page 63, used the same combination and evidently thought that he was doing so for the first time. Both these publications antedate Massee's 'British Fungous Flora', Vol. IV, 1895, where he first used the combination, by several years. Smith [1931], Böning [1933] and several others still give credit, however, to Massee for this combination which, on the evidence presented here, is not his; Butler and Bisby [1931] correctly cite De Bary as the authority.

Distribution of the fungus within the host.

In January 1932 the affected plants were uprooted and the distribution of the fungus within them was traced in two ways: by cutting sections to examine them for the presence of the fungus in the tissues of the stem and by attempting to isolate the fungus from various parts of the stem by tissue fragment culture. Of the ten plants uprooted at that time the canker in each one started from six to seven and a half feet from the ground. The stems were sectioned at intervals of every six inches from the foot of the stem up to the end of the pallid colour of the rotted portion. The mycelium was observed to extend only to two or three inches below the whitish patch and below that the fungus was completely absent. When surface sterilised tissue fragments from the stems were placed on agar slants the fungus grew out of those that were from a region just below the rotted portion and it was completely absent from the rest of the plant below.

This showed that the fungus had invaded the plant from somewhere above ground and not through the roots. In order to test this, a more thorough test was carried out in the season of 1932-33. The fungus was grown in giant cultures on sterilised paddy and by the end of June a large quantity of sclerotia was available together with the mycelium. These sclerotia and the mycelium were thickly spread in a plot about 14 × 4 feet and covered over with a thin layer of soil. The plot

was kept watered every now and then and seed of the green-stemmed variety of *Patwa* was sown on the 10th of July.

Germination was good and there were no seedling deaths. Beginning with the 1st of August, plants were carefully uprooted every two or three weeks and both the roots and the stem were sectioned and examined to find out the presence of any fungus. Tissue fragment cultures were also made. Up to the 6th of December no fungus could be detected in the plants. On the 6th of January 1933 the disease however became manifest on the axis of the inflorescence of these as well as the other plants.

An examination of the affected plants was again made and the fungus was seen only in the region of the rotted portion and for about two to three inches below it but not in the main body of the stem. This showed conclusively that the disease was not systemic and that it was air borne. Similar examination of the plants was done in the season of 1933-34 with confirmatory results.

In the season of 1932-33 a watch was placed to see where the first lesion was produced. Field examination of the plants showed that the first part to be affected is the dead corolla or calyx and the fungus then finds its way to the main axis through the pedicles. This happens in January. It has been already stated that the sclerotia need a cool temperature, that is a temperature of or below 22°C., for the development of apothecia. The fact that the first appearance of the disease is only in January when atmospheric temperatures are around 22°C. and that it appears at a distance of six to eight or more feet above ground strongly indicated that the infection may be through the ascospores which may be wafted by the wind to the dead calyces. It is also possible that bees and other insects which visit the flowers for the honey in the glands on the calyx or for pollen carry the infection to that height. Both the microconidia and the ascospores can be the source of infection, for it would not be possible for the sclerotia to reach that height. Inasmuch as the microconidia are abortive and do not germinate, suspicion strongly rested on the ascospores. Furthermore the microconidia are produced only in culture media and their production did not seem to be conditioned by temperature though it did seem to depend on the food supply.

INFECTION EXPERIMENTS.

At the time the infection experiments were planned it was not known that it did not take place through the soil and the roots. The following plan of experiments was therefore laid down :

1. To determine whether infection is through the soil.
2. To determine whether it is through injured or uninjured tissues.

3. To determine whether the fungus is capable of infection when prevailing temperatures are not cool, that is, before December-January.
4. To determine whether the sclerotia, the mycelium and the ascospores each can or cannot cause infection.

1. To determine whether infection is through the soil.

The experiments reported in the previous section conclusively showed that infection is not caused directly by the sclerotia and the mycelium in the soil. Observation made in 1933-34 confirmed these results.

2. To determine whether it is through injured or uninjured tissue.

Experiment.—Six healthy plants growing in 12-inch pots and about 2·5 to 3·0 feet high were selected and vigorously growing mycelium from a potato-dextrose agar tube was placed in the axils on the leaf-petiole with the main axis about two feet from the ground. The mycelium was wrapped by means of wet cotton and cellophane wrappers. The cellophane covering helped in retaining the moisture. Six other plants were similarly wrapped with wet cotton and cellophane but there was no mycelium. The infection was done on the 3rd of September 1932. The cotton wool and cellophane wrappers were removed after four days. Even though the weather was wet, the infected plants were sprayed with distilled water through a De Vilbiss atomiser three times a day. No infection of the plants had taken place even by the end of November.

Experiment.—The above was repeated with one dozen plants in the field but infection was done on the 10th of September 1932. No infection of the plants took place.

Experiment.—The above was repeated with ten potted plants in September 1933 and the previous result was confirmed. Field plants also gave a negative result.

Experiment.—While the previous experiments were done with plants where the fungus material was placed on uninjured tissue, the above tests were repeated but with this difference that the tissue was slightly injured at the place where the mycelium was placed. The tests were made in September 1932 and again in 1933. None of the infected plants showed any canker or other signs of the disease by the end of November following infection.

The above experiments showed that if the Hibiscus plants are infected in September when prevailing temperatures are rather high, infection of plants does not take place even when the tissues are injured.

3. To determine whether the fungus is capable of infection at other times than September.

Both potted and field plants, with and without injuring the tissues were infected following the plan adopted in previous experiment. The results obtained are tabulated below :—

TABLE III.

Results of infection experiments to determine whether infection can take place in October, November and December.

Date	Potted or field plants	Injured or uninjured	No. infected	No. showing disease
1932				
October 3 . .	Potted . .	Injured . .	7	None in six weeks
Do. . .	Field . . .	Do. . .	10	Ditto
October 5 . .	Potted . .	Uninjured . .	4	Ditto
Do. . .	Field . . .	Do. . .	8	Ditto
November 6 .	Do. . . .	Injured . .	10	Ditto
Do. .	Do. . . .	Uninjured . .	10	Ditto
December 2 .	Do. . . .	Injured . .	9	Canker in three weeks
Do. .	Do. . . .	Uninjured . .	7	Ditto

The experiments recorded in Table III were done with plants sown on the 6th of July both in the pots and in the field. The infecting material was either sclerotia or mycelium and as ascospores were not available they could not be used in 1932. Experiments using ascospores were done, however, in November 1933 with the following results.

Experiment.—On November 12th, 1933, ten plants in the field were selected and in the axils of the pedicels with the main axis, ascospores were placed together with a bit of the apothecial disc. On another five plants only a suspension of the ascospores in sterile water was placed in the axils. On a third batch of five plants, the tissue was slightly injured before placing the ascospore suspension. A thin layer of moist cotton was placed on the infected part in each case and this was wrapped with cellophane. On the fourth day the cellophane wrapper and the cotton

wool were removed. On the 23rd of November 1933 the pallid colour became manifest which spread rather slowly at first but rapidly later and on the 12th of December there were large cankers on all but the control plants which had received similar treatment but without any infecting material.

Experiment.—The above experiments were repeated but the ascospores used were from the *S. sclerotiorum* received from Dr. H. H. Whetzel. In plants where the tissues were uninjured, no infection appeared even on the 25th of January 1934 but where the tissues had been injured, the lesion became manifest on the 19th of November. It spread rather slowly but never caused such a large canker as in the previous case.

These experiments showed that ascospores of the Hibiscus Sclerotinia can cause infection even as early as November both on injured and uninjured tissues of the plants. The ascospores of *S. sclerotiorum* from the U. S. A., were unable to infect Hibiscus plants unless the tissues were injured.

4. To determine whether sclerotia and mycelium can or cannot cause infection.

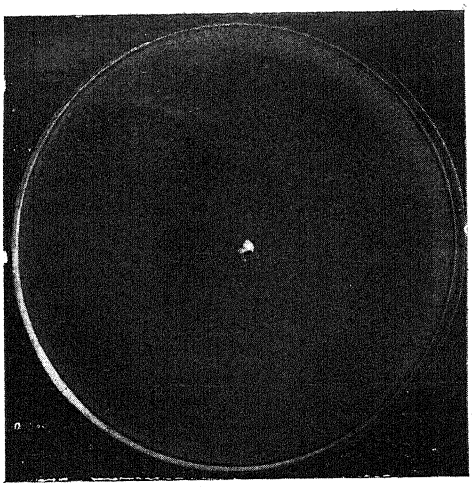
It remained now to determine whether the sclerotia and mycelium can infect the host plants when prevailing temperatures are low. That the ascospores were capable of infection was already proved.

Experiment.—On the 11th of January 1933, seven plants in 12-inch pots were infected with the mycelium, three plants being injured and four left uninjured before applying the infective material. The pots were placed away from places where natural infection was likely to occur. Five plants were placed near these pots as controls. None of the controls showed any infection. None of the uninjured plants showed any infection. But plants receiving the mycelium on injured tissues were all affected and the disease was observed on the 27th of January, sixteen days after the plants were infected.

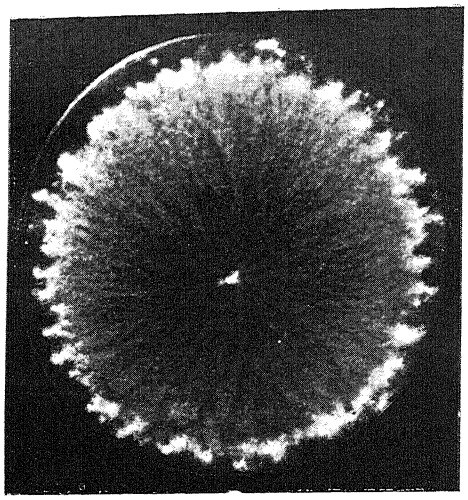
Experiment.—Similar tests were repeated with sclerotia. In this case one more batch of five plants but infected with cut sclerotia was also included. Infection was produced only in those cases where the sclerotia were cut and not in other cases. On the 6th of February when the plants were examined, it was noticed that the uncut sclerotia had not even put forth any mycelial growth.

These and other experiments recorded in Table IV showed that the mycelium is capable of causing the disease in winter if it is placed on plants at a place where the tissues are injured. But if the tissue is uninjured, it cannot cause the disease. Sclerotia were also capable of causing infection provided they were given a start by cutting them open. But if they are uncut, there is not evidently enough of some stimulus to make them grow.

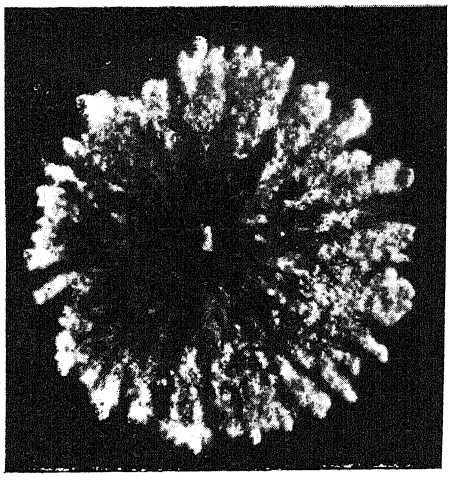
PLATE LIII.



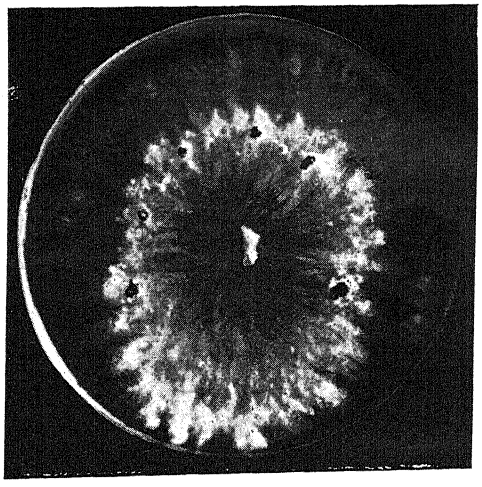
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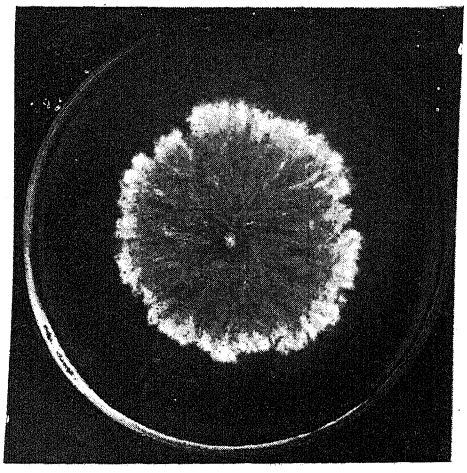
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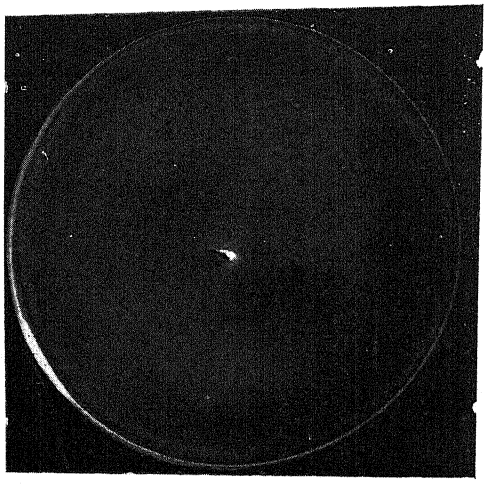
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Figs. 11 to 16. Petri dishes showing growth of fungus at 8·0, 20·0, 22·5, 25·0, 27·5 and 32·5°C.

TABLE IV.

Record of infection experiments done in January with Mycelium.

Date	Potted or field plants	Injured or uninjured	No. of plants	No. attacked
1933				
January 11	Potted	Injured	3	3
Do.	Do.	Uninjured	4	0
Do.	Do.	Do.	4	0
January 12	Field	Injured	20	19
January 14	Do.	Do.	18	18
Do.	Do.	Do.	24	18
Do.	Do.	Uninjured	10	0
		Do.	30	30
January 16	Do.	(Ascospores)		

In all cases where the fungus caused infection of the test plants, the usual symptoms that are observed in the fields were reproduced and the mycelial strands and sclerotia on the surface of the stem and within the pith were also noticed. The fungus was re-isolated from these plants by tissue fragment culture and by incubating the sclerotia on the nutrient medium.

TEMPERATURE RELATIONS OF THE FUNGUS.

During 1930-31 and the succeeding years the disease first appeared in the early part of January and the infection experiments further showed that the fungus was incapable of producing the disease in August, September, October and November. The ascospores can infect the plants in November but they are usually not available under field conditions earlier than December. Temperature therefore seemed to exercise a good deal of influence on the growth of this fungus and experiments to determine the cardinal temperatures for growth were started in 1932.

Nine incubators were available for carrying on these tests. In the winter months they can be accurately set to the different required temperatures, variation being less than half a degree in twenty-four hours. The tests were conducted in Petri dishes of 110 mm. diameter on Coons', cornmeal and Kotila's agars. Linear rate of growth was measured from the third day onwards by measuring the diameter of the colonies and then averaging them. All the usual precautions regarding sterilisation, depth of medium, etc., were of course taken. There were twelve Petri dishes for each temperature but due to contamination some had to be rejected. The readings are therefore based on seven, six or five replications as the case may be for each temperature. The data are recorded in Table V.

TABLE V.

Growth of Hibiscus Sclerotinia at different temperatures on Coons', cornmeal and Kotila's agars.

Replicates	8·0°			15·0°			17·5°			20·0°			22·5°			25·0°			27·5°			30·0°			32·5°		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	4	0	0	..	..	46	48	..	..	110	69	..	110	78	62	74	46	92	55	50	20	20	22	26	0	0	0
2	0	0	0	..	..	39	54	..	..	45	56	..	110	66	57	74	55	69	90	43	13	36	22	30	0	0	0
3	4	0	0	..	..	35	49	..	..	110	64	..	110	65	47	44	50	83	72	44	26	25	21	15	0	0	0
4	3	0	0	..	..	27	61	..	..	102	58	..	106	70	58	105	51	85	48	46	44	15	19	18	0	0	0
5	3	0	0	..	..	32	70	..	..	110	53	..	105	71	54	110	56	90	60	42	30	18	23	13	0	0	0
6	4	0	..	..	..	..	58	..	..	106	69	..	110	59	..	105	46	..	59	49	..	23	18	..	0	0	..
7	..	0	..	..	..	..	..	..	..	..	63	..	..	74	..	..	48	..	..	50	..	..	20	..	..	0	..
Totals .	18	0	0	..	..	179	340	..	..	583	432	..	651	483	278	512	352	419	384	324	133	141	145	102	0	0	0
Average .	3	0	0	..	..	36·0	57·0	..	..	97	62	..	108	69	56	85	50	84	64	46	27	24	21	20	0	0	0

A = Coons' agar, B = Cornmeal agar, C = Kotila's agar.

A reference to the data recorded in Table IV will show that the best growth was promoted in all the three media in those Petri dishes that were placed in the incubator registering 22·5°C. It further became manifest that Coons' agar was the best of the three culture media that were tried. When we speak of the influence of temperature on the growth of a fungus we really mean the temperature of the fungus hypha and it is this that directly influences its rate of growth. It is conventional however to assume that the temperature of the hyphæ follows closely the temperature of the medium which again is same as the temperature of the surroundings, in this case the temperature of the incubator. The diameter increments then at 22·5° can be considered as optimum for the growth of this fungus, defining the term ' optimum temperature ' as " that temperature at which there is best growth during a given time period," for without this qualification of time the term ' optimum temperature ' does not have any meaning as pointed out by Blackman [1905].

While the tests carried on above were on solidified agar media, one more on a liquid medium was also tried. The medium was that of Coons. Two hundred and fifty cubic centimeter flasks were used in the tests and in each fifty cubic centimeters of the medium were placed. After sterilisation the flasks were inoculated with a suspension of the mycelium in water and the test was done in triplicate. Temperatures below 20°C. were not unfortunately available. The fungus was incubated for fourteen days after which the flasks were removed from the incubators and further growth was stopped by adding to each flask ten cubic centimeters of formaldehyde. The contents of each flask were then filtered through weighed filter papers which were then dried to constant weight in an oven registering 96° C. The data are recorded in Table VI where each of the weight given is the average of the three flasks.

TABLE VI.

Weight in milligrams of Hibiscus Sclerotinia at different temperatures.

Temperature (°C.)	Weight of growth (mg.)
20·0	308·3
22·5	377·2
25·0	133·6
27·5	96·1
30·0	27·5
32·5	10·6

It will be noticed that again the best growth is registered at a temperature of 22·5°C. While in the case of solid media no growth took place at 32·5° the fungus grew slightly at that temperature in the liquid medium. The minimum for growth then seems to be somewhere below 8° while the maximum for growth is at 32·5°C. after which growth ceases.

A test was then conducted to see at what temperature the sclerotia are killed. They were placed in a beaker and this was left in a hot air oven registering 60°C. Ten sclerotia each were removed after one, four, eight, twelve and sixteen hours. These were washed with hydrogen peroxide and placed on potato-dextrose agar slants. Those that had been exposed for one and four hours were found to put forth growth after seven days while the others were evidently dead as they did not grow even after a month. Joshi [1924] immersed the sclerotia of this fungus for five minutes in water at different temperatures and found that a temperature of 48-50°C. killed the sclerotia. But they can evidently stand a higher temperature when dry.

A growth-temperature curve is given below :—

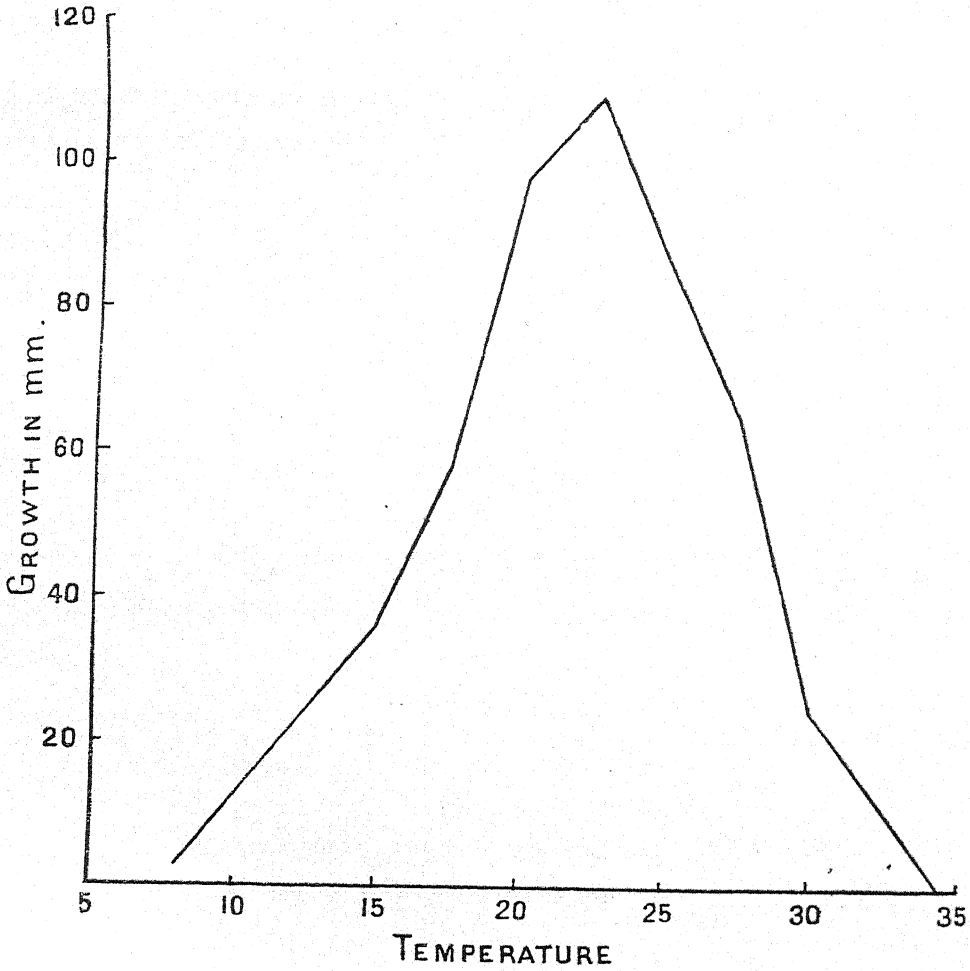


Fig. 1. Growth-temperature curve of Hibiscus Sclerotinia.

Dissemination of the disease.

The disease can over-summer in several ways. The mycelium present in the affected tissue may be a source of danger if it can survive the summer heat. Sclerotia formed on and within the stem can carry over the disease to the next season. Sclerotia have also been found in the seed collected from a plot where the disease was present. In order to be sure whether the fungus can persist in this manner, several experiments were conducted.

Mycelium in the tissues.—Tissue fragment cultures of affected stems collected in February 1931 by Dr. W. McRae were made in the following October. One hundred and ten cultures were in all made but the fungus did not grow in any of the tubes showing that the mycelium is not viable after eight months. Confirmatory results were obtained in 1932 and 1933 also.

Sclerotia in the tissues of the stem.—Sclerotia formed on and in the stem were placed on potato-dextrose agar slants after surface sterilisation. Of the one hundred and four sclerotia that were grown, 68 or 65·3 per cent. were viable. The sclerotia can therefore oversummer on or within the host. Dr. McRae had stored in a test-tube some sclerotia in March 1931. In October they were planted on potato-dextrose agar in the usual manner. There were 43 sclerotia but none of these germinated showing that if they are stored in test-tubes, they dry up and cannot survive the summer heat.

Sclerotia in the seed.—Seed collected from a plot where the disease was present were examined for the presence of sclerotia. Random samples of seed were taken and the number of sclerotia in each sample was determined. Results are given in Table VII.

Table VII.

Percentage of sclerotia in seed and their viability.

Total seed	Sclerotia	Per cent. present	Per cent. viable
1,244	14	1·12	64·0
1,024	13	1·36	66·6
552	13	2·35	72·1
849	11	1·30	57·8
2,134	26	1·20	67·3

Per cent viability was determined by planting them on an agar medium, incubating at favourable temperatures and noting how many had germinated. It will be noted that the seed can carry an appreciable amount of infection and that the sclerotia are mostly viable.

Control measures.

In devising control measures the following facts had to be kept in mind:

1. That the seed carry infection as sclerotia.

2. That the disease does not appear until the cool temperatures of January have set in.

3. That on uninjured tissue only ascospores can cause infection and that these do not occur in nature before December.

Sclerotia and seed are large enough in size to permit hand picking of the former and ridding the seed of infective material to a very great extent. Of the mechanical means to separate the sclerotia from the seed, if the healthy seed had a specific gravity other than that of the sclerotia, then it is possible to devise means to separate the two. But specific gravity determinations of the healthy seed and the sclerotia indicated that the former had a specific gravity of 0·836 while the latter had 0·880. As the two values are so close, it is hardly possible to use these means.

As four years' observations indicated that the disease does not appear before the first week of January, efforts were made to see if it would be possible to escape it by inducing the crop to mature earlier. Ordinarily it is harvested for seed in the middle of February but if the harvesting could be hastened by at least two weeks, then the effects of the disease could be very much minimised. With this end in view, five lines each were sown on the first and fifteenth of April, May, June and July. By earlier sowing it was hoped that the crop could be induced to mature earlier. The early-sown plants grew immensely tall and vigorous but all of them flowered almost at the same time as the late sown ones and set seed at the same time also. No hope lay along that direction therefore. Mention may be made here of the fact that the early-sown plants set very few seed and the stems also suffered from some wet rot with which a species of Colletotrichum, Fusarium and *Rhizoctonia bataticola* were associated.

Infection experiments indicated that the sclerotia and mycelium in the soil cannot cause direct infection of the plants and that the sclerotia form potential danger only if they form the apothecia. In order to see if the disease could be controlled by ploughing the sclerotia deep into the soil, they were buried at a depth of nine, six, three and one inch in the soil placed in pots. A large number of sclerotia were used for each depth and the soil was kept moist by watering occasion-

ally. Sclerotia developed into apothecia in January but only from those pots in which they were placed on the surface or had been buried just an inch below. In the other pots the sclerotia did not form apothecia. Good ploughing of the land in order to turn in the sclerotia is therefore a good practice in such plots where the disease had appeared the previous season. If the seed is sown also at a depth of more than an inch, sclerotia would have a little chance of causing any harm.

Eight types of *Hibiscus sabdariffa* and several hybrids between these types are grown on the Botanical area. A survey of the disease made in 1932-33 and 1933-34 indicated that most of the types and hybrids were affected. Hope of obtaining resistant types from the existing material was slight. It would be possible to obtain a mixed population of seed from elsewhere and then see if resistant types can be secured. The control measures then may be summarised as follows :

1. Hand pick the seed and separate out all sclerotia.

2. Plough the land deep to bury all the sclerotia that may lie on the fields and sow the seed deeper than one inch so that the sclerotia, if any, may not have a chance of putting out apothecia.

3. Look out for early maturing and resistant types.

Summary.

Hibiscus sabdariffa suffers from a stem-rot due to *Sclerotinia sclerotiorum* (Lib.) de Bary. The disease occurs on the seed crop only in January and the following month. In August, September, October and November the sclerotia and mycelium are unable to bring about the disease on the host plant but in December and January, they can infect the host provided the tissues are injured at the time of applying the infection. Ascospores can bring about the disease on injured or uninjured tissues in November and the succeeding months but these ascospores are not available before the cool temperatures, that is 22°C. and below, have set in, for it is only then that the perfect stage of the fungus is formed.

Best growth of the fungus also takes place when the temperatures are cool, that is about 22°C. Minimum for growth of the fungus seems to be at 8° and below, and maximum at 32·5°C. The sclerotia are not killed even by a temperature of 60°C. if dry heat is applied but if they are immersed in water for five minutes at 48-50°C. the sclerotia are all killed.

Hand-picking the sclerotia from the seed and ploughing them rather deep in the soil are some of the measures to control the disease. A lookout for resistant and early-maturing types should also be kept so that the disease can be brought under control.

ACKNOWLEDGMENTS.

Grateful thanks of the writer are due to Dr. W. McRae, Imperial Mycologist and Director, who made the preliminary observations on this disease, for entrusting me with the problem and the material he had collected and for encouragement and guidance. Thanks are due to Drs. H. H. Whetzel and G. B. Ramsey for supplying authentic cultures of the Sclerotinias. Maulvi Mahomed Taslim of this section rendered useful help in the field experiments and in making the counts.

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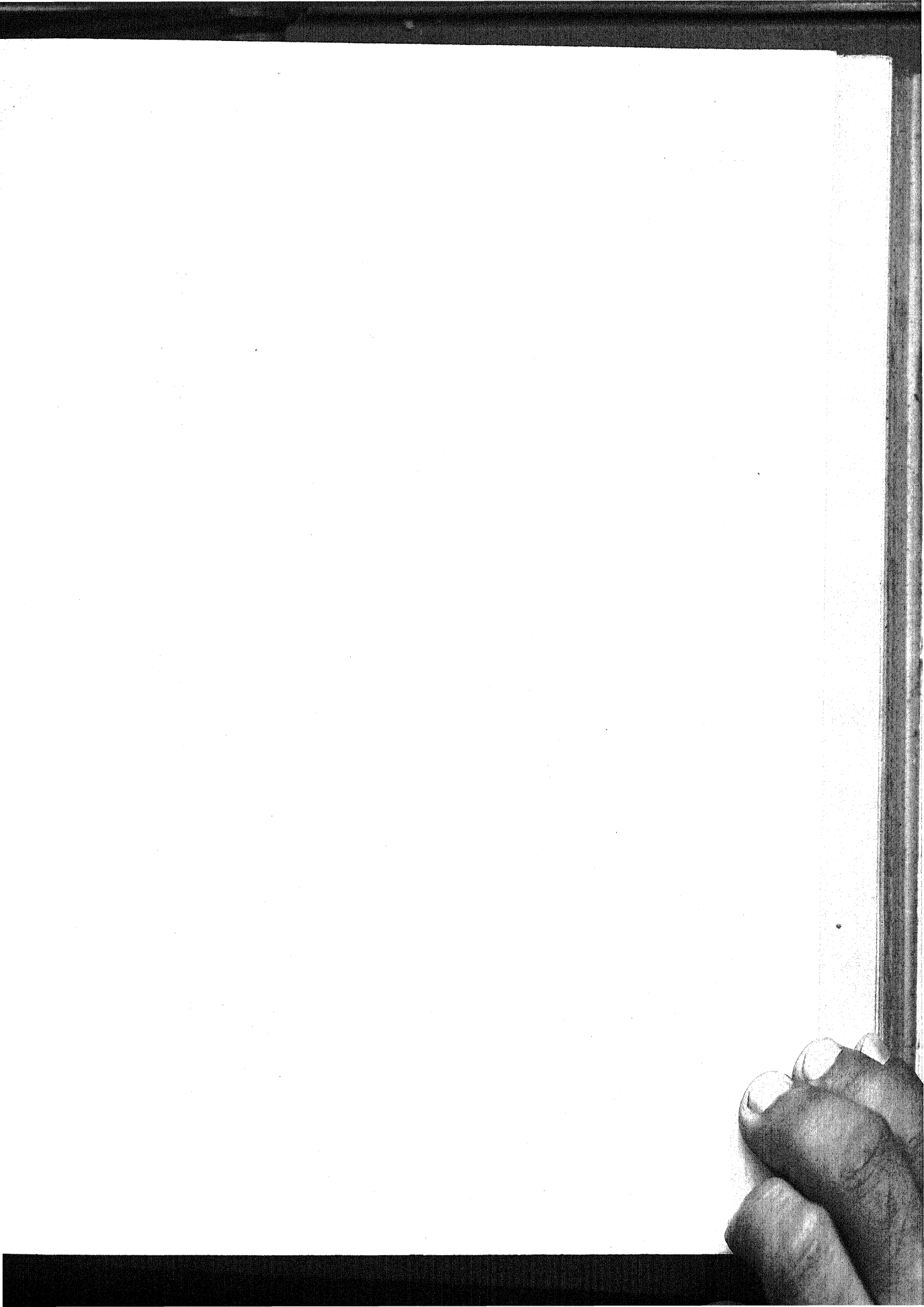
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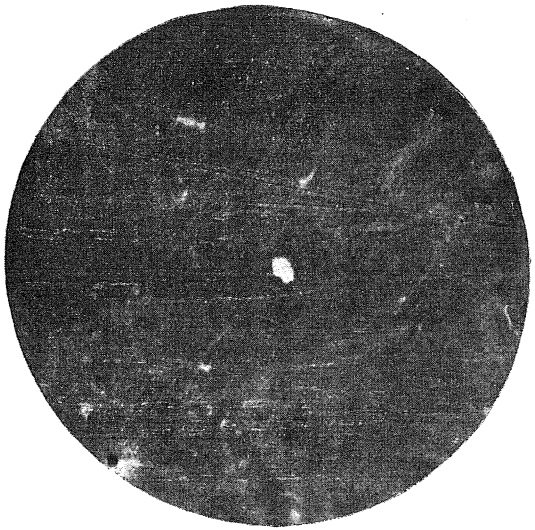


Fig. 1. Culture of *Corticium rolfsii* showing the basidial stage (white portion in centre) and sclerotia.

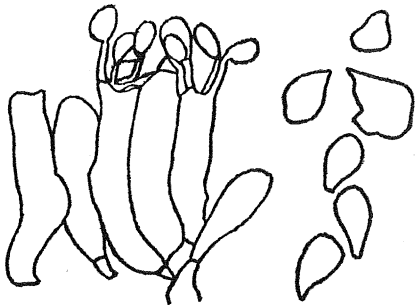


Fig. 2. Basidia with sterigmata and basidiospores.

PERFECT STAGE OF *SCLEROTIUM ROLFSII* SACC. IN CULTURE.

(Preliminary Announcement.)

BY

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(With Plate LIV)

The chief characteristic of the fungus *Sclerotium rolfsii* Sacc. is the absence of any sexual (perfect) or asexual spores. Two investigators have reported a reproductive stage of this fungus which they obtained in culture media. Goto [1930] secured the perfect stage on onion agar in Formosa, Japan, and Curzi [1932] obtained it on potato-glucose agar in Italy. The fungus is now placed on the basis of this new information in the hymenomycetous genus Corticium of the group Basidiomycetes.

Attempts to induce the perfect stage in isolates that are under study at Pusa were until recently unsuccessful. Both onion agar and potato-glucose agar made according to the directions of Goto and Curzi, respectively, gave negative results. Various modifications were then tried but without success. A medium was finally developed which has given good results. This medium is made as follows :—

Cut onions	100·00 grams
Asparagin (Merck)	0·25 ,,
Proteose peptone (Bacto)	0·50 ,,
Bacto agar	15·00 ,,
Distilled water to make	1000·00 c.c.

Onions are steamed in a requisite quantity of water in the Koch steriliser, strained, and to the clear liquid the rest of the ingredients are added. The mixture is then boiled in a double boiler until the agar is dissolved. Volume is restored, the medium is tubed and then sterilised at 10 lb. pressure in the autoclave for 15 minutes.

Both flask and test-tube cultures have proven of value. It takes forty to forty-five days for the hymenial structure to appear and it can be distinguished by its white colour and texture (Plate LIV, fig. 1). High temperatures, 30-31°C., seemed to

favour hymenial production, for the basidial stage is rather rare in cultures placed at lower temperatures. According to Higgins [1927] the optimum temperature for the growth of *S. rolfsii* is between 30 and 32°C.

The following isolates have so far developed the perfect stage :—

No. 10. Isolated from wilted cotton plants by writer at Dharwar in 1931.

No. 29. Isolated from betel-vines (*Piper betle* L.) at Bassein near Bombay and sent by Mr. J. S. Malelu in 1932.

No. 101. Isolated by writer from diseased potato plants in 1933.

No. 124. Isolated by writer from diseased sugarcane plants brought from Taliparamba in 1933.

In mounting the hymenial tissue for microscopic examination on a slide Amann's lacto-phenol mounting medium is superior to plain water in which the sporidia get detached. The basidial stage is white and stands out prominently from the rest of the mycelium. It is always aerial, inclined to be a little dense and dendroid in shape. The basidia are rather short and thick and densely aggregated into a crust which may measure 6 to 12 mm. They are hyaline, clavate and septated at the basal end and bear two, three or four sterigmata which are 3 to 5μ in length. A majority of the basidia do not bear sterigmata or the spores and evidently remain sterile. About fifty basidia were measured and the extreme and mean measurements are : Length 16·5 to 39·0μ with a mean of 24·5 $\pm$ 3·6μ. Breadth 4·2 to 6·1μ with a mean of 5·7 $\pm$ 0·43μ.

The basidiospores are hyaline, smooth-walled and usually obovate or globose. When only two basidiospores were borne, they tended to be slightly cylindrical and sometimes irregular in shape. Fifty basidiospores have been measured and the extreme and mean measurements are as follows :

Length 4·9μ to 9·4μ. Mean $=6{\cdot}8\pm0{\cdot}067\mu$.
Breadth 2·6μ to 7·1μ. Mean $= 4{\cdot}9\pm0{\cdot}057\mu$.

These measurements indicate that the Indian strain of *S. rolfsii* (No. 10) agrees with Goto's strain *R* 11 and with Curzi's potato strain which Curzi calls *Corticium rolfsii*. Whether Curzi's name *C. rolfsii* can be retained for the Indian strain or whether it is some other new species can only be determined by a detailed comparative study which is in progress. Tentatively it is placed in that species. As several sclerotial fungi are commonly confused under the name *S. rolfsii*, it is possible that detailed studies may reveal that not all the strains now under study are referable to one single species.

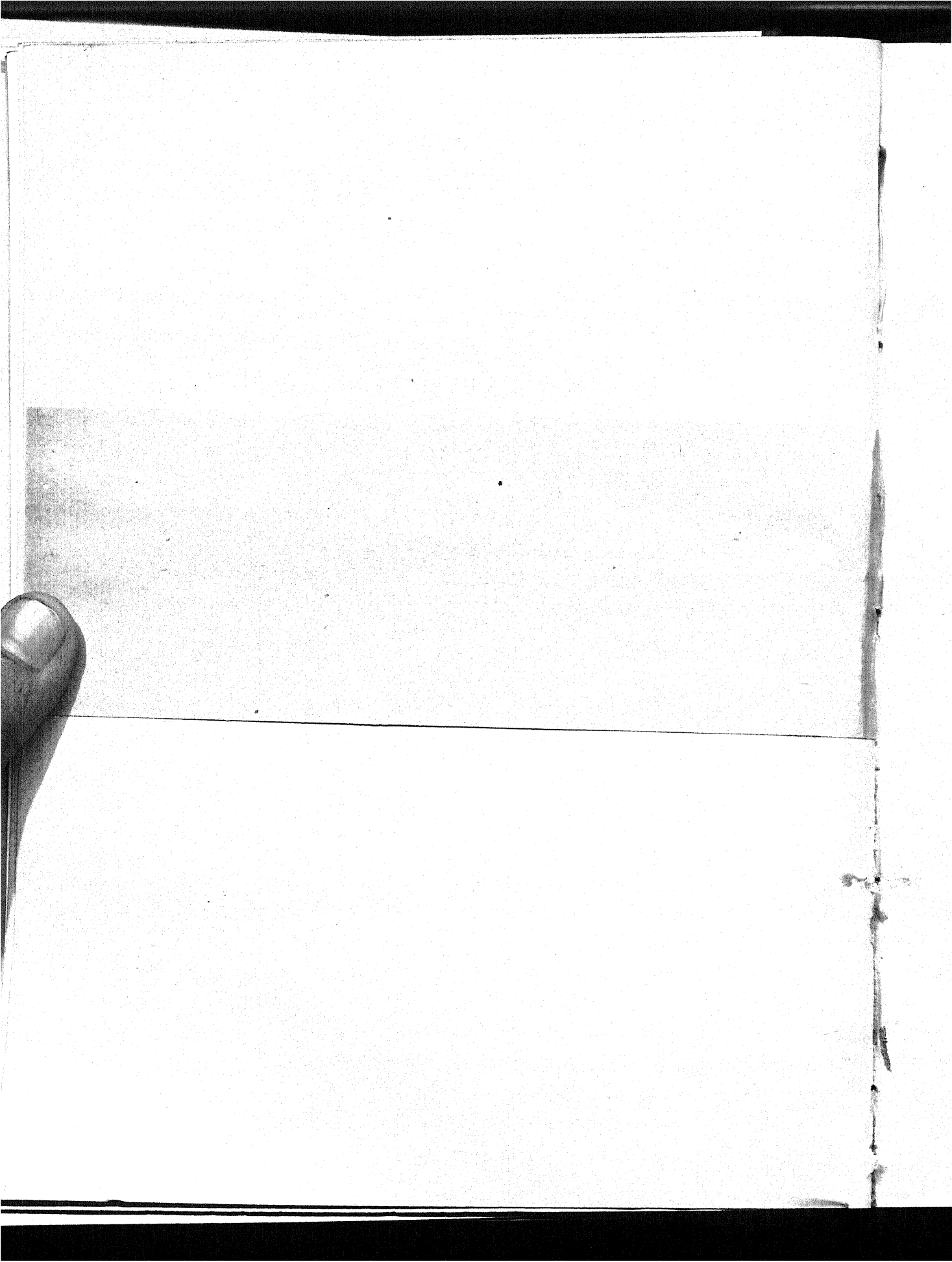
Finally in order to determine the genetic connection between the sporidial and sclerotial stages, monosporidial cultures were obtained using Watson's micro-manipulator. The method followed was essentially the same as that described by Hanna [1928]. Out of thirty-eight sporidia that were picked and planted on onion-proteose-peptone agar, only eleven grew successfully and formed typical sclerotia. The mycelial characters as well as the sclerotial characters of these daughter cultures were like those of the parent cultures. In two tubes the hymenium had formed also and basidia and basidiospores were observed. It was clear that the sporidial stage is genetically connected with the sclerotial stage and that there is no heterothallism, at least in the strain that was studied, *viz.*, No. 10.

SUMMARY.

Sclerotium rolfsii Sacc. that was under study at Pusa formed the sexual stage on a special medium containing onions, asparagin and proteose peptone. It appeared forty to forty-five days after the tubes had been inoculated and at temperatures which promoted optimum growth of the fungus, *viz.*, 30-31°C. The fungus agreed with the description of *Corticium rolfsii* of Curzi and is tentatively placed in that species.

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ORIGINAL ARTICLES

EFFECT OF MOSAIC ON THE TONNAGE AND THE JUICE OF SUGARCANE IN PUSA, PART IV.

BY

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(Received for publication on 19th April 1934)

In continuation of the last three seasons' experiments [McRae, 1931, 1932; McRae and Subramaniam, 1933] thirty-six plots each 5×56 yards were laid down in adjacent pairs in the order mosaic-free, mosaic-infected, mosaic-infected, mosaic-free and so on. The cane was planted on the 28th and 29th January 1933. The area was good, light land suitable for growing sugarcane and was adjacent to last year's series of plots. Rape cake and acid superphosphate at the rate of ten maunds and two maunds per acre respectively were applied at the time of planting. For the mosaic-infected plots setts were cut from canes whose leaves had the mosaic markings at the time of planting while for the mosaic-free plots setts were cut in the main sugar growing area of the farm from canes that from frequent observations during the growing season were known to be free from mosaic disease.

During the season 1933-34 a very small amount of infection spread to the mosaic-free plots, one clump being observed to have the disease in each of seven plots. This was in agreement with the results of another plot where a row thirty yards long of each of forty-five varieties of mosaic-free cane had been planted alternately with a row of mosaic-infected Co. 213 and during the season three varieties became infected. Co. 303 had one, Co. 313 two and Saretha one clump showing the characteristic mottling of the leaves. The spread of mosaic during this season was remarkably low.

On the 27th March, 16th April and 13th May 1933, the number of shoots was counted and the percentage calculated on the number of buds on the setts planted.

The percentage of shoots that were above ground after eight, eleven and fifteen weeks from planting was as follows.

TABLE I.

Percentage germination.

Plot No.	*	1	4	5	8	9	12	13	16	17	20	21	24	25	28	29	32	33	36	Mean
	F	1	4	5	8	9	12	13	16	17	20	21	24	25	28	29	32	33	36	
	M	2	3	6	7	10	11	14	15	18	19	22	23	26	27	30	31	34	35	
27-3-33		30	23	27	30	28	29	29	27	27	25	23	36	33	32	34	36	36	37	30
		23	27	28	30	32	34	26	29	27	28	26	22	25	25	23	23	25	25	26
19-4-33		38	37	37	41	38	39	37	32	32	30	27	39	40	37	38	42	41	42	37
		29	34	45	40	42	42	31	34	32	34	32	30	32	30	28	28	29	30	33
13-5-33		65	71	76	94	86	100	84	74	77	76	70	93	89	91	96	90	84	69	83
		51	60	82	93	100	100	67	77	87	80	84	78	83	74	71	69	49	53	75

* F represents mosaic-free and M mosaic-infected plots.

Between the last two counts the plots had to be irrigated with about two inches of water in which a little crude oil emulsion was mixed in order to reduce the attack of termites. During this interval a considerable number of tillers appeared so that the last count does not represent germination alone. The previous count on 19th April 1933, however, represents germination after eleven weeks from planting. The difference of the means is statistically significant, odds 60:1, showing that germination in the field was less by four per cent. in the mosaic-infected cane. After planting the new season's crop, pieces of cane each containing an eye, were placed in moist sawdust in partial shade at temperatures, ranging from 64° F. minimum to 100° F. maximum. Of 500 eyes from mosaic-free cane 437 germinated and of the same number from mosaic-infected cane 419 germinated. The difference is 3·6 per cent. and this is confirmatory of the indication that the mosaic-infected cane had a slightly less germinability than the mosaic-free cane.

As was done previously a record was kept of the main facts regarding the presence of insects that destroy cane and on the whole it shows that the attack of insect pests was fairly evenly distributed over the plots except for top shoot and stem borer.

In the hot weather the cane was attacked by the top shoot borer, *Scirpophaga nivella*, and by termites. In early and late June the bored and dead shoots were removed and the percentages calculated on the number of shoots in each plot are shown in Table II.

TABLE II.

Percentage of borer and termite attack in June.

F	M	Borer						Termites					
		F			M			F			M		
Plots		First count	Second count	Total	First count	Second count	Total	First count	Second count	Total	First count	Second count	Total
1	2	3·5	1·4	4·9	4·2	1·2	5·4	0·2	0·5	0·7	0·5	0·3	0·8
4	3	2·7	0·2	2·9	4·8	0·2	5·0	0·5	0·7	1·2	0·3	0·0	0·3
5	6	2·2	1·0	3·2	1·9	1·0	2·9	0·4	0·5	0·9	0·5	0·3	0·8
8	7	2·3	0·4	2·7	2·6	0·8	3·4	0·1	0·2	0·3	0·6	0·1	0·7
9	10	2·4	0·5	2·9	2·0	0·7	2·7	0·3	0·5	0·8	0·3	0·1	0·4
12	11	1·4	0·1	1·5	1·6	0·5	2·1	0·2	0·1	0·3	0·3	0·3	0·6
13	14	3·8	0·2	4·0	3·9	0·6	4·5	0·2	0·2	0·4	0·4	0·3	0·7
16	15	1·8	0·5	2·3	2·1	1·0	3·1	0·2	0·2	0·4	0·1	0·2	0·3
17	18	1·7	0·4	2·1	2·9	0·3	3·2	0·3	0·2	0·5	0·4	0·2	0·6
20	19	5·8	0·5	6·3	2·8	0·5	3·3	0·2	0·2	0·4	0·4	0·3	0·7
21	22	2·3	0·4	2·7	2·2	0·5	2·7	0·2	0·3	0·5	0·2	0·2	0·4
24	23	1·5	0·4	1·9	2·2	0·3	2·5	0·3	0·1	0·4	0·1	0·2	0·3
25	26	1·6	0·6	2·2	1·8	0·6	2·4	0·3	0·1	0·4	0·2	0·2	0·4
28	27	1·3	0·4	1·7	2·1	0·2	2·3	0·3	0·1	0·4	0·3	0·2	0·5
29	30	1·5	0·5	2·0	4·3	0·8	5·1	0·2	0·2	0·4	0·4	0 2	0·6
32	31	2·2	0·3	2·5	2·8	0·5	3·3	0·3	0·1	0·4	0·6	0·4	1·0
33	34	2·2	0·9	3·1	3·2	0·6	3·8	0·2	0·2	0·4	0·6	0·4	1·0
36	35	1·6	0·2	1·8	2·9	0·9	3·8	0·3	0·3	0·6	0·5	0·3	0·8
Mean .	.	..	..	2·82	..	..	3·42			0·53			0·58

The difference between the means is statistically significant in the case of borer but not in that of termites. Odds 35:1.

During the four months from March to June egg-masses of *Scirpophaga nivella* and *Pyrilla* species were collected regularly and the numbers are given in Table III.

TABLE III.

Egg-masses collected from 11th March to 23rd June.

Plot F	Plot M	*Scirpophaga nivella* F 1st	F 2nd	F 3rd	F 4th	F 5th	F 6th	F 7th	F Total	*Scirpophaga nivella* M 1st	M 2nd	M 3rd	M 4th	M 5th	M 6th	M 7th	M Total	*Pyrilla* species F 1st	F 3rd	F 5th	F 6th	F 7th	F Total	*Pyrilla* species M 1st	M 3rd	M 5th	M 6th	M 7th	M Total
Count																													
1	2	13	5	1	..	1	1	1	22	3	13	5	..	..	3	1	25	..	5	4	3	6	18	1	3	4	..	4	12
4	3	6	23	2	..	2	..	3	41	14	21	..	..	..	..	..	35	..	7	..	..	1	8	2	6	6	..	4	18
5	6	10	12	1	..	..	..	..	23	8	12	..	..	..	..	2	22	3	9	6	..	5	23	3	9	4	1	7	24
8	7	9	15	2	..	..	..	..	26	9	10	..	..	2	..	..	21	2	7	4	..	5	18	3	4	..	..	3	10
9	10	2	11	..	..	..	..	2	41	20	13	1	..	1	..	3	38	3	5	3	..	4	15	2	4	2	2	5	15
12	11	8	25	..	..	2	..	..	35	4	9	2	..	1	..	..	16	3	11	1	..	5	20	5	10	3	..	4	22
13	14	14	9	3	..	..	..	..	26	5	4	..	..	4	..	3	16	2	9	3	..	8	22	..	3	5	..	2	10
16	15	15	6	..	..	..	1	2	24	4	9	..	..	..	..	1	14	6	7	1	..	7	21	3	7	1	1	5	17
17	18	7	12	..	..	2	2	1	24	4	1	..	..	..	..	..	5	1	4	..	..	1	6	1	6	3	1	3	14
20	19	6	2	..	..	..	..	3	11	3	1	1	..	..	..	..	5	4	5	4	2	6	21	2	5	3	1	4	15
21	22	4	3	..	..	..	..	..	7	3	4	..	..	1	1	1	10	3	6	3	..	2	14	1	6	1	..	2	10
24	23	3	5	..	..	2	..	2	12	2	1	..	..	..	..	3	6	1	9	4	1	6	21	3	7	7	..	4	21
25	26	4	2	..	..	..	..	3	9	1	1	..	..	1	..	..	3	3	9	2	..	3	17	3	13	3	1	4	24
28	27	7	2	..	..	..	..	..	9	2	1	..	..	..	..	1	4	3	10	5	..	3	21	2	8	4	2	1	17
29	30	9	3	..	2	..	..	1	15	1	1	..	..	1	..	2	5	2	9	4	..	3	18	1	5	3	..	4	13
32	31	2	6	..	..	1	..	..	9	2	2	..	..	1	..	1	6	2	14	1	..	5	22	3	4	2	..	1	10
33	34	13	4	..	..	..	..	..	17	..	2	..	..	..	..	..	2	3	16	3	..	2	24	2	8	4	..	7	21
36	35	4	3	..	..	..	..	..	7	3	3	..	..	2	..	1	9	2	15	2	3	5	27	1	8	2	1	4	16
Mean		..	..	..	..	..	..	..	20	..	..	..	..	..	..	..	13·4	..	..	..	..	..	18·6	..	..	..	..	..	16

After the 23rd of June no more egg-masses were collected as the plants were becoming tall, the weather was rainy and the ground soft. *Pyrilla aberrans* Kby., *P. pusana* Dist., and *P. perpusilla* Wlk. occur in Pusa but no attempt was made to distinguish to which species the various egg-masses belonged.

The difference between the means is statistically significant in the case of *Scirpophaga nivella* odds very high but not in that of *Pyrilla*. The collection of these egg-masses has probably saved the two sets of plot from greater damage by these insects.

No damage was done by other cane diseases nor by animals. During harvest the plots were examined under the direction of the Imperial Entomologist and Table IV gives the percentage of insect infestation.

TABLE IV.

Percentage of insect infestation at harvest.

Plots		F				M			
F	M	Top shoot borer	Stem borer	Root borer	Termites	Top shoot borer	Stem borer	Root borer	Termites
1	2	13·4	5·9	17·2	11·3	23·1	6·5	12·9	11·6
4	3	13·7	6·9	14·7	13·8	29·8	7·6	16·8	7·6
5	6	13·5	7·3	11·7	16·0	27·8	8·2	18·3	6·7
8	7	14·1	7·7	14·5	7·2	25·4	5·3	14·2	7·2
9	10	12·6	6·7	12·6	6·5	23·3	7·4	11·7	3·8
12	11	12·0	6·5	12·5	5·8	21·2	8·0	13·6	5·6
13	14	10·4	6·7	10·3	6·1	15·0	7·6	11·0	4·6
16	15	13·2	4·3	17·4	5·7	15·6	7·3	14·1	7·0
17	18	13·6	8·2	14·7	9·7	12·5	8·8	14·6	7·9
20	19	14·1	7·4	16·1	4·7	14·0	7·7	16·3	8·4
21	22	13·1	7·4	13·2	6·9	11·8	7·7	11·3	6·1
24	23	11·2	7·4	12·8	9·5	16·3	6·5	11·6	11·2
25	26	11·7	6·4	12·4	9·4	10·4	7·0	12·4	11·7
28	27	11·5	7·4	12·1	5·1	12·3	8·4	12·3	6·1
29	30	10·8	7·1	12·2	4·2	13·8	8·3	10·9	6·3
32	31	11·0	7·9	11·5	6·2	11·9	9·2	14·3	5·0
33	34	10·7	7·0	11·3	4·8	14·4	7·6	10·3	11·2
36	35	9·5	6·1	9·9	9·2	12·6	7·8	12·4	13·3
Mean.		12·2	6·9	13·2	7·9	17·2	7·6	13·3	7·8

The differences between the means are statistically significant in the cases of top shoot borer (odds 180:1) and steam borer (odds 42:1) but not of root borer and termites. In March 1934 seven random samples of 25 canes per sample were taken on the Farm from mosaic-free Co. 213 cane bored by top shoot borer and seven more

similar samples from similar unbored cane. The weight of the samples from the former were 42·5, 42·5, 43, 42, 45·5, 44 and 43·5 lbs. and from the latter 61, 63, 69·5, 58·5, 51·75, 56·5 and 50 lbs., the respective means being 43·3 and 58·6 lbs. The difference 15·3 lbs. is statistically significant (odds very high) indicating a loss of 26 per cent. in weight in the cane attacked by top shoot borer. The Assistant Entomologist gave us the following weights of six samples, each containing twenty-five canes, of Co. 213 that had been attacked by stem borer at harvest 46·75, 48·75, 41·5, 39·5, 44·5 and 40 lbs., the mean being 43·5 lbs. The difference between it and 58·6 lbs., the mean of the unbored cane as stated above was 15·1 lbs. and is statistically significant (odds very high) indicating a loss of 25·8 per cent. in weight of cane attacked by stem borers. These facts will be taken into consideration later in the discussion.

Samples of cane were taken for analysis just before harvest from the 19th to 24th February when the cane was considered to be ripe. The sample from each plot consisted of five yards of cane taken at random from each of the five rows and the cane was crushed in the small, bullock-driven, three-roller, iron mill. The analysis of juice was furnished by the Chemical Section and the details are as follows :—

TABLE V.

Samples taken from 19th to 24th February, 1934.

Plot		Weight of canes		Weight of juice		Percentage extraction		Brix		Sucrose		Glucose		Purity	
F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M
1	2	368	306	253·5	205·5	68·8	67·1	18·00	18·49	15·42	15·73	·58	·53	85·67	85·06
4	3	346·5	340	230	227·5	66·4	66·9	19·08	18·64	16·61	16·03	·48	·56	87·04	86·01
5	6	336	275	230·25	183	68·5	66·5	18·34	17·98	15·66	15·06	·57	·64	85·41	83·75
8	7	309	339	207·25	230	67·1	67·8	18·33	18·20	15·76	15·26	·51	·69	85·99	83·86
9	10	390	327	263·5	220	67·5	67·3	17·81	17·60	15·09	14·84	·64	·70	84·74	84·31
12	11	396	301	268	197·5	67·6	63·9	17·28	17·77	14·47	14·95	·70	·76	83·73	84·12
13	14	380	324	266	218·5	70·0	67·5	16·49	18·04	13·49	15·44	·97	·53	81·81	85·60
16	15	294	290	195	194	66·3	66·9	18·70	17·67	16·25	14·81	·40	·58	86·88	83·81
17	18	366	352	252	237	68·8	67·3	18·69	17·84	16·15	14·94	·45	·67	86·41	83·77
20	19	448	309	312	212·75	69·6	68·8	17·31	18·23	14·42	15·36	·73	·56	83·29	84·24
21	22	326	320	222	219	68·1	68·4	18·04	17·47	15·42	14·24	·44	·82	85·46	81·50
24	23	314	296	211	199	67·2	67·2	18·07	17·74	15·42	14·98	·53	·58	85·31	84·42
25	26	425	287	290	196	68·2	68·3	17·77	17·61	15·02	14·81	·56	·60	84·53	84·12
28	27	365	310	294·25	213	68·3	68·7	18·61	18·37	15·94	15·54	·45	·52	85·64	84·60
29	30	325	326	223	218	68·6	66·9	17·28	18·45	14·30	15·85	·62	·46	82·75	85·91
32	31	401	314	283	217	70·5	69·1	18·11	18·37	15·27	15·47	·49	·45	84·30	84·21
33	34	364	338	253	231	69·5	68·3	17·80	17·23	15·02	14·23	·57	·58	84·38	82·58
36	35	252	252	170	171	67·5	67·8	19·78	18·04	17·14	15·15	·36	·55	86·63	83·98
Mean .	.	355·8	311·4	245·7	210·5	68·25	67·48	18·08	17·98	15·38	15·15	·56	·60	84·99	84·21
Difference .		44·42		35·22		·77		·1		·23		·04		·78	

The edges were cut away leaving 36 plots each 5 by 50 yards and they were harvested between the 5th and the 10th March. The weights in maunds of stripped cane are as follows :—

(1 maund =82·28 lbs.)

TABLE VI.

Weight in maunds of stripped cane.

Plots		F	M	Plots		F	M
1	2	45·05	36·71	20	19	44·55	37·75
4	3	42·21	36·45	21	22	41·23	40·88
5	6	40·88	37·46	24	23	46·24	40·11
8	7	44·35	39·35	25	26	44·82	38·77
9	10	42·72	41·43	28	27	44·31	39·53
12	11	48·06	39·90	29	30	44·56	40·34
13	14	45·94	36·95	32	31	45·57	36·54
16	15	40·39	34·88	33	34	45·15	34·47
17	18	42·74	39·79	36	35	40·30	32·97

The mean of F is 43·84 and the mean of M is 38 maunds. The difference, 5·84 maunds or 13·3 per cent. is significant.

The figures to determine the statistical significance of the difference in yield between the two series of eighteen pairs of plots are summarised below :—

TABLE VII.

Statistical constants.

Co. 213	Mean difference	Standard deviation	Mean difference / Standard deviation	Odds
Weight of cane	5·84	2·65	2·2	Very high
Percentage of juice to cane	0·77	1·5	0·63	70:1
Calculated juice per plot	4·30	2·1	2·05	Very high
Brix	0·10	0·78	0·04	0
Sucrose	0·24	1·13	0·21	4:1
Glucose	0·04	0·17	0·023	0
Purity	0·78	2·03	0·38	14:1

The differences in weight of cane, percentage juice to cane, and calculated juice per plot alone were statistically significant. In the three preceding years the difference in infestation by insects was not statistically significant, consequently there was no need to estimate the loss caused by these factors. But this season the differences both for top shoot borer and stem borer were statistically significant so the difference due to borer has to be subtracted from 13·3 per cent. According to the figures in Table IV, 12·2 per cent. and 17·2 per cent. respectively of the canes were attacked by top shoot borer and 6·9 and 7·6 per cent. respectively by stem borer. The loss in weight of cane bored by these two insects has already been found by experiment to be approximately 26 per cent. The loss due to both borers together is accordingly 4·96 per cent. in the mosaic-free and 6·45 per cent. in the mosaic-infected plots. The recorded mean weight of cane in the mosaic-free plots, 43·84 maunds, represents 95·04 per cent. of the expected weight, if the cane had not been bored; similarly 38 maunds represent 93·55 per cent. in the mosaic-infected plots. If there had been no borer attack the means of the weights of the two sets of plots would have been 46·12 and 40·62 maunds respectively. The difference 5·5 maunds represents 11·9 per cent. and is approximately the loss in weight due to mosaic disease. This season then in the mosaic-infected plots there was a loss of about 12 per cent. of stripped cane and ·77 per cent. less juice was extracted. The quality of juice as measured by brix, glucose, sucrose and purity was, however, not affected.

On reviewing the results of the four seasons' experiments one sees that no factor that has been measured has varied consistently in all four tests except calculated juice from cane—

Co. 213	1931	1932	1933	1934
	Per cent.	Per cent.	Per cent.	Per cent.
Weight of cane	—4·6	—14·8	*	12
Percentage juice to cane	*	*	—2·47	—·77
Calculated juice	—4·8	—15·4	—4·0	—13·8
Brix	—·81	—·58	*	*
Sucrose	—·99	—·67	*	*
Glucose	*	*	—·08	*
Purity	—1·56	*	*	*

The weight of cane has been less in the mosaic plots three times giving an average of —8·2 per cent. per season. Percentage juice to cane, brix and sucrose have each been less twice, while glucose and purity have been less once. So far there is a probability that in Co. 213 mosaic disease reduces the weight of the cane and the juice from the cane but there is no clear indication that the quality of the juice is affected.

For the information of the ordinary person who glances at this note we should like it to be clearly understood that we have been dealing with a comparison between wholly mosaic-infected Co. 213 cane and mosaic-free Co. 213 cane. Now a field of wholly mosaic-infected Co. 213 cane does not exist in North Bihar outside our experimental plots. According to the results of the investigation on the intensity of mosaic infection in 1933 it was found that the average amount of infection was only ·58 per cent. in Co. 213. A twelve per cent. loss in weight of stripped cane in a wholly mosaic-infected crop would represent a loss of only ·07 per cent. on the ordinary crop as grown in North Bihar or a decrease of 7 maunds in every 10,000 maunds which so far is insignificantly small.

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EFFECT OF MOSAIC DISEASE ON THE TONNAGE AND THE JUICE OF SUGARCANE IN PATNA.

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The effect of the mosaic disease in reducing the tonnage of sugarcane at Pusa has been intensively studied by McRae [1931, 1932] and McRae and Subramaniam [1933, 1934]. Their results indicate that during three years there has been a significant decrease in yield of stripped cane due to this disease while in one year there was no such difference. The work done at Pusa in North Bihar does not show whether similar results will be got under different climatic and soil conditions and as it is rather important to have an answer to this question. Mr. Sethi, Director of Agriculture, Bihar and Orissa, on the suggestion of Dr. McRae, Imperial Mycologist and Director, Pusa, kindly agreed to place land on the Patna Farm at the writers' disposal for carrying on a comparative test.

Patna is on the southern side of the Ganges and has a climate which is different from that obtaining at Pusa. It is on the whole warmer in summer and during the monsoon, and the winter also lasts for a much shorter time than it does at Pusa. On the Pusa Farm the cane is grown without irrigation while regular irrigation is the practice at Patna. Furthermore, the soil at Pusa is a sandy loam but on the Patna Farm it is a heavy, almost black clay. During the monsoon the soil at Patna swells up and assumes a spongy consistency but as the land begins to dry up, it contracts and cracks are formed on the soil surface. A chemical analysis of the soil at Patna and Pusa is given for comparison in Table I.

TABLE I.

Chemical analysis of Patna and Pusa soil.

	Patna	Pusa
	0—6″ Block C East of road	0—6″
Loss on ignition	5·427	2·92
Insoluble silica	71·678	56·40
Ferric oxide	7·108	2·70
Alumina	11·024	2·80
Lime	1·162	18·30
Magnesia	1·311	1·65
Manganese oxide	*nil*	·07
Potash	1·923	·51
Soda	·248	·19
Carbonic anhydride	..	14·33
Sulphuric anhydride	..	·02
Phosphoric anhydride	·119	·11
	100·000	100·00
Nitrogen	·064	·067
Available phosphoric anhydride	·0064	·003
„ potash	·0082	·0109

Material and methods.

The land that was available was of good tilth and well suited for growing sugarcane. It was manured at the rate of 200 maunds of farmyard manure per acre and at the time of planting the setts it received an additional dose of 'nisiphos' at the rate of one maund per acre. The land was divided into twenty plots, each 44 yards long and 5 yards broad and planting was done on the 2nd of February 1933. The cane selected for the test was Co. 213. The canes were planted in the order

mosaic-free, mosaic-infected, mosaic-infected, mosaic-free and so on. Planting was done eye to eye and the shoots subsequently came up freely in all the plots.

Mosaic-infected canes for the test were obtained from Pusa where the setts were selected at the time of harvest from plants whose leaves showed the usual symptoms of the mosaic disease. The mosaic-free canes were carefully selected from the crop grown on the Patna Farm from plants whose leaves had no mottling.

The crop was examined periodically during the season. It was noticed that a slight amount of infection had spread from the diseased plots to only three clumps of two of the mosaic-free plots, so the mosaic-free plots remained substantially unaffected.

The shoots that had appeared above ground on the 27th April and 27th May were counted and the figures in Table II show the number of shoots above ground after the twelfth and sixteenth weeks.

TABLE II.

Percentage of shoots above ground relative to the number of eyes planted.

Plot number		27th April 1933		27th May 1933	
*F	M	F	M	F	M
1	2	14	16	81	69
4	3	18	20	90	90
5	6	16	22	100	90
8	7	18	17	100	84
9	10	16	16	97	82
12	11	18	15	97	83
13	14	18	13	100	75
16	15	16	13	94	79
17	18	19	13	100	91
20	19	18	13	100	100

* F=mosaic-free and M=mosaic-infected.

The mean emergence after twelve weeks in the mosaic-free plots and the mosaic-infected plots was 16·9 and 15·9 per cent. respectively and the mean emergence after

sixteen weeks was 95·9 and 84·3 per cent. respectively. In order to find out whether the difference in emergence of the two setts of plots was statistically significant z-test was applied. In the case of the reading taken on 27th April 1933, it was found that there was no statistical significance, the value of the odds being very low (3:1). But the difference in mean emergence on the later date did have statistical significance, odds being 1999:1. This difference was due to a greater degree of tillering in the mosaic-free canes than in the mosaic-infected ones.

While McRae and Subramaniam had to encounter difficulties arising out of insect infestation in their trials at Pusa, fortunately in this test few insects attacking canes were observed and they did a comparatively insignificant damage.

The crop was also free from other fungous diseases excepting smut which appeared in April 1933. A careful examination revealed that smut occurred only in the mosaic-free plots and that mosaic-infected canes were free. Enquiries regarding the previous history of the cane showed that the crop from which the mosaic-free setts had been obtained for planting had suffered from smut in the previous season and that other plots on the farm were suffering similarly from smut. The setts of diseased cane obtained from Pusa came from a crop that was free from this malady. The smut-infected canes were however carefully rogued out and the record of plants removed is given in Table III.

TABLE III.

Smut-infected cane plants removed from mosaic-free plots.

Plot number	1	4	5	8	9	12	13	16	17	20
No. of shoots removed .	27	24	23	24	25	32	31	43	47	32

These canes were taken out in April while the plants were still young. There was time for their places to be taken in whole or in part by other tillers. It is impossible to estimate the effect of this loss accurately and the difference between the means of the two sets of plots would be increased, if at all, by less than 1·6 per cent. as calculated from the samples which we consider typical of the plots.

Samples of canes were removed at random from each line in a plot for chemical analysis between the 11th and the 14th March 1934. The samples from each plot consisted of 2 yards of cane from each of 5 rows and the cane was crushed in a bullock-driven, three-roller, iron mill. The weight of the samples and of the juice, the percentage weight of the juice to cane and the brix readings obtained are

recorded in Table IV. Unfortunately the juice could not be analysed in the last ten samples due to unforeseen illness of the Chemical Assistant's family.

TABLE IV.

Plot		Weight of cane in maunds		Weight of juice in maunds		Percentage weight of juice to cane		Brix corrected	
F	M	F	M	F	M	F	M	F	M
1	2	2·15	1·30	1·37	0·86	64·0	66·3	19·18	19·28
4	3	1·50	1·87	1·00	1·27	66·6	68·0	19·40	19·30
5	6	1·98	1·25	1·36	0·84	68·6	65·1	19·10	18·21
8	7	1·71	1·48	1·11	0·98	65·3	66·3	18·88	19·01
9	10	2·07	1·33	1·39	0·88	66·9	66·0	19·48	18·68
12	11	1·52	1·53	0·97	0·96	64·0	62·7	18·41	18·81
13	14	1·64	1·45	1·04	0·93	63·4	64·1	18·48	18·72
16	15	2·10	1·90	1·36	1·21	64·6	63·6	18·78	19·37
17	18	1·38	1·10	0·91	0·73	66·0	66·4	19·18	18·38
20	19	1·39	1·10	0·89	0·72	65·7	65·0	18·87	19·08
Mean . .		1·74	1·43	1·14	0·93	65·51	65·35	18·97	18·88
Difference .		—·31		—·21		—·16		—·09	

Finally the canes were harvested on the 17th of March 1934. Edge effect was eliminated by cutting off two yards of cane growing at the ends of the rows and five lines of cane at the sides of the two end plots thus leaving plots 5×40 yards in extent. The weight of stripped cane from each plot is shown in Table V.

TABLE V.

Weight in maunds of stripped cane.

F	M	F	M	F	M	F	M
1	2	35·44	28·18	12	11	37·44	29·85
4	3	32·05	29·95	13	14	38·29	31·20
5	6	35·46	27·85	16	15	37·82	31·55
8	7	34·38	26·49	17	18	38·58	30·26
9	10	35·82	28·49	20	19	34·66	27·10

The mean of the mosaic-free plots is 35·99 while that of the mosaic-infected is 29·09 maunds. The difference 6·90 maunds or 19·17 per cent. is statistically significant.

It will be noted from the data recorded in Tables IV and V that the weight of cane and of juice, the brix reading and the weight of stripped cane are in favour of the mosaic-free plots. The figures for determining the statistical significance of the differences in the various factors measured are summarised below :—

Co. 213	Mean difference	Standard deviation	Ratio	Odds
Weight of cane	6·9	1·7	4·0	Very great.
Percentage juice to cane	0·16	1·5	0·1	1·59:1
Calculated juice to cane	4·57	1·36	3·3	Very great.
Brix	0·09	0·51	0·18	2:1

Thus the difference in weight of cane and calculated weight of juice to cane alone are statistically significant.

Conclusions.

The mosaic-infected cane had 19 per cent. less weight of stripped cane and the calculated juice was less by 19·4 per cent. The spread of the disease was very small. The results secured at Patna are conformable with those reported by McRae and Subramaniam at Pusa where during the present season the weight of stripped cane was less by 12 per cent. and the calculated weight of juice was less by 13·8 per cent. in the mosaic-infected plots.

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HYDROGEN ION CONCENTRATION AND THE INTAKE OF NITROGEN BY RICE PLANT.

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INTRODUCTION.

The problem of nitrogen nutrition of the rice plant has attracted the attention of physiologists since Kellner [1884] made the first observation that the rice plant needed ammoniacal nitrogen in the early stages of growth and nitrate nitrogen in the later stages of growth. Kellner [1884] was followed by Nagaoka [1904], Daikuhara [1905], Aso and Bahadur [1906], Krauss [1907], Trelease and Paulino [1920], Kelley [1911, 1914], and they all confirmed his result. Espino [1920] and Trelease [1920] carried on extensive investigations on the salt requirements of the rice plant and showed the importance of ammonium sulphate on the growth of the rice plant.

The greater beneficial effect of ammoniacal nitrogen than that of the nitrate nitrogen has been attributed by Kinoshita [1894-1897] to the greater rapidity of the conversion of ammonium ion into asparagine while by Willis and Carrero [1923] to the toxic influence on the plants of the basic residues of nitrate salts which interfere with the absorption of iron. In all the work done on the salt requirements of the rice plants, the absorption of the essential elements is determined by making analysis of the whole plant at different stages of growth, and the relative importance of ammoniacal nitrogen and nitrate nitrogen is determined by the yield and growth of the treated and untreated plants. But so far no quantitative data about the intake of ammonium ions and nitrate ions by the rice plants at different stages of growth from the known concentrations of these ions in the external media were obtained by direct chemical analyses of the latter. The entry of ions inside plant cells is governed by many hitherto not much understood physico-chemical laws, and in order to understand them, such direct determinations of the ions taken in from the

external media are absolutely necessary. It is now well known that two ions of a salt are not equally absorbed and the position of equilibrium between the inside and the outside of the plant may vary with different substances and in different species.

This gap in the knowledge about the quantitative data regarding the absorption of ammonium and nitrate ions from the solutions of known concentrations is filled recently by Dastur and Malkani [1933]. They have shown that the absorption of ammonium ion progressively decreases and the absorption of nitrate ion progressively increases as the rice plant ages. These facts hold good for single salt solutions containing ammonium or the nitrate ion, for a solution containing both the ions and for ordinary culture solutions. There is unequal absorption of the ammonium and sulphate ions, the former being absorbed in greater amounts than the latter and it renders the external solution more acidic as there is no exosmosis from the roots of positively charged ions to replace the excess of ammonium ions absorbed [Dastur and Malkani, 1933].

The fact that the absorption of ammonium ion decreases with the age is true of all ammonium salts while the nitrate-ion absorption increases from all salts containing nitrates.

Dastur and Malkani [1933] offer no explanation of this unique behaviour of the rice plant towards the absorption of nitrogen. They suggest that the permeability of protoplasm may be responsible for the absorption of one kind of nitrogen at one stage and of the other at the other stage of growth.

The most important factor that affects the physical and chemical behaviour of protoplasm is the hydrogen ion concentration. There is mutual reaction between plants and their external media. The plants affect the hydrogen ion concentration of the culture solution and the changes in the hydrogen ion concentration of the nutrient solutions react upon the plants. These actions and reactions between plants and the hydrogen ion concentration of the external solutions are not properly understood, and these reactions are affected by many other factors. The maximum growth of seedlings of different species occurs at different ranges of pH values as shown by Tarr and Noble [1922]. Hoagland [1926] has also shown that slight acidity is not injurious to various types of plants but pH values of 9 and above are injurious to crop plants. As a result of further work on the growth of crop plants by various workers like Salter and McIlvaine [1917], Cole [1922], Hixon [1920], Arrhenius [1922] and many others it has been observed that there are two values of hydrogen ion concentration where the growth of plants reaches its maximum values. The double maximum curve of growth is an interesting feature as such a curve is obtained when the viscosity, osmotic pressure and electrical conductivity of certain

colloids are plotted against the pH value of the solution and it has been found that the minimum between the two maxima is located at the iso-electric point of true substances. It is just likely, as suggested by Robbins [1923], that there is an iso-electric point for the plant tissue which corresponds to pH value at which there is the minimum point of growth between the two maxima. Robbins [1923] obtained a double maximum curve with a minimum between, for water absorption in the case of potato tissue in contact with solutions of different hydrogen ion concentrations and his results indicated that an ampholitic protein was the chief cause of it with its iso-electric point at the minimum point of water absorption. The iso-electric point of a plant tissue and its effect upon water absorption give rise to such double maximum growth curves of plants when plants are grown in media of different pH concentrations. It is also likely that other physiological functions are similarly affected. It is therefore very likely that the absorption of water and ions depends on the variations of the pH of the cell sap from that of the iso-electric point of proteins in the roots of the rice plants. The greater absorption of ammonium ions during the early stages and of nitrate ions in the later stages, may be due to the changes going on in the hydrogen ion concentration of the cells of the roots. It is therefore undertaken to study the changes going on in the hydrogen ion concentration of the roots and leaves of the rice plants at different stages of growth and under different treatments and to determine the pH at which the proteins of the protoplasm are at their iso-electric point.

I. Investigation.

The investigation is divided into three parts. The first part deals with the determinations of hydrogen ion concentration of the soil and of the roots and leaves of the rice plants manured with (1) ammonium sulphate, (2) sodium nitrate and (3) a mixture of ammonium sulphate and sodium nitrate on equal total nitrogen basis and (4) of untreated rice plants. The determinations are made at regular intervals as far as possible during the life cycle of the plants. The mixture of ammonium sulphate and sodium nitrate is used because it is shown by Dastur and Pirzada [1933] that a mixture of these two nitrogen containing salts is more beneficial in its effects upon the growth of the rice plants than any one single salt.

The second part deals with culture experiments—rice seedlings are grown in the solutions of (1) sodium nitrate, (2) ammonium sulphate and (3) a mixture of ammonium sulphate and sodium nitrate. The pH values of the culture solutions are determined before the seedlings are kept in them, and after the seedlings had remained in them for a week. The period of one week is selected as it was determined by Dastur and Malkani [1933] that at the end of seven days the equilibrium between the external concentration and the internal concentration of

ions of the salts is reached. Different concentrations of these three culture solutions are used.

The third part deals with the determination of the iso-electric points of the principal proteins of the roots and leaves of the rice plants. The basis of the experiments is the comparison of the rates of diffusion of some easily determined ion, from living tissues kept in solutions of different hydrogen ion concentrations, and the chloride ion seemed convenient for this purpose.

Method.

The most accurate method available for the measurement of hydrogen ion concentration is the electrometric method. Throughout the present work, therefore, this method has been employed.

The temperature was maintained at 32°C. by means of an air thermostat heated by an automatic electric heater and regulated by means of a toluene regulator. The air inside the box was kept moving by means of a small fan. The temperature remained constant with a variaton of $\pm 0{\cdot}05$°C. This particular temperature is selected because the variations of the atmospheric temperature of Bombay lie between 29°C. and 31°C.

The hydrogen electrode was of platinum foil coated evenly with platinum black. The electrode vessel was type illustrated by Clark [1920] with a bubbler on the side to prevent evaporation of the solution. The electromotive force of the solutions was measured by Poggendorf's compensation method using the type of portable potentiometer manufactured by Cambridge Instrument Company.

The apparatus was tested before use with two standard buffer solutions (i) $N/20$ sodium borate and (ii) universal Buffer mixture powder of B. D. H. and was found to be accurate. The barometric pressure was noted at each observation and the observed electromotive force was corrected for pressure from a graph plotted from the data given by Clark [1920].

Rice seedlings of the Columbia variety No. 42 were obtained from the Rice Research Station, Karjat, and were transplanted on 16th July 1932 in the beds of the garden of the Royal Institute of Science. Usual care was taken to use uniform samples of soil for all beds and it was also ascertained that the soil did not lack in any of the necessary elements. On the 5th of August 1932 the manuring was done as follows :—The first bed was manured with ammonium sulphate, the second with sodium nitrate, the third with the mixture of ammonium sulphate and sodium nitrate and the fourth was not at all manured. The fourth was meant to serve as a control. There were duplicate sets of each of 4 treatments. After allowing a fortnight for the plants to establish themselves and to grow, the

first group of plants was taken out for examination on the 30th July. The roots were thoroughly and very carefully washed with tap water, the roots and leaves were separated from the body of the plants and the sap was extracted by a method described by Ingalls and Shive [1931].

The soil surrounding the roots was taken up in paraffined glass dishes and was spread thinly to dry up speedily in air, free from laboratory fumes. It has seemed more expedient for the present however to study the air-dried soils. The samples then were powdered and were passed through a 1-m.m. sieve. Fifteen grms. of the soil were taken into a hard glass test tube and 30 c.c. of distilled water were added to it. The soil was allowed to remain in contact with water for 24 hours to ensure complete soaking and thorough wetting of the soil particles. Then the tube was violently shaken about 50 times and the sample was centrifuged for at least twenty minutes. The clear supernatant fluid was drawn off with a pipette and put to test. The electrode vessel containing the soil solution was shaken 50 to 60 times per minute with the help of the low power motor.

Table I gives the pH values of the unmanured soil and of the roots and leaves of the rice plants grown in it. Tables II-IV give the pH values of the soil and of the roots and leaves of the rice plants manured with ammonium sulphate, sodium nitrate and a mixture of the two salts respectively.

TABLE I.

pH values of the soil, root and leaf of unmanured rice plants, at different stages of growth.

Date 1932	pH of the soil	pH of the root	pH of the leaf	pH of the inflorescence	Remark
5th July . . .	6·97	..	..	..	Before transplantation.
30th „ . . .	6·44	6·18	5·82	..	
15th August . . .	5·92	3·44	6·15	..	
24th „ . . .	6·84	..	6·21	..	
30th „ . . .	6·38	3·47	5·92	..	Heavy rains.
7th September . .	..	2·94	..	..	„
9th „ . .	5·92	3·27	6·05	..	„
14th „ . .	6·84	5·35	6·03	..	„
1st October . .	6·66	5·50	5·97	..	„
3rd „ . .	6·97	5·49	5·92	..	„
12th „ . .	7·04	5·92	6·05	6·23	Cloudy.
18th „ .	6·99	5·09	6·11	5·42	„
24th „ . .	6·51	4·59	5·87	4·45	Raining.

TABLE II.

pH values of the soil, root and leaf of rice plants manured with ammonium sulphate at different stages of growth.

Date 1932	pH of the soil	pH of the root	pH of the leaf	pH of the inflorescence	Remark
5th July . . .	6·91	..	..	..	Before transplantation.
3rd August . .	5·92	6·16	5·82	..	Before manuring.
27th ,, . .	6·81	4·23	6·05	..	After manuring, no rains.
2nd September . .	6·35	4·26	6·02	..	Heavy rains.
13th ,, . .	6·51	5·87	5·85	..	Raining.
17th ,, . .	6·58	..	..	..	
21st ,, . .	5·92	7·52	5·25	..	
22nd ,, . .	6·21	6·35	5·73	..	
23rd ,, . .	6·53	6·33	5·72	..	
4th October . .	6·88	5·97	5·80	..	Rains.
5th ,, . .	6·74	..	5·90	..	,,
7th ,, . .	7·16	5·85	6·05	6·03	
14th ,, . .	6·91	5·82	6·11	5·82	Raining.
27th ,, . .	6·51	4·92	5·22	..	All starchy.

TABLE III.

pH values of soil, root and leaf of rice plants manured with sodium nitrate at different stages of growth.

Date 1932	pH of the soil	pH of the root	pH of the leaf	pH of the inflorescence	Remark
5t l July . . .	6·91	..	..	..	Before transplantation.
1st August . . .	6·41	6·18	5·80	..	Before manuring.
25th ,, . . .	7·01	3·77	6·11	..	After manuring.
31st ,, . . .	6·53	3·80	6·13	..	Slight rains.
10th September . .	6·63	7·01	5·69	..	,,
15th ,, . .	7·06	7·01	5·72	..	
27th ,, . .	6·66	6·18	5·77	..	Rains.
28th ,, . .	7·07	5·72	..	..	,,
11th October . . .	6·23	3·97	6·08	6·41	,,
17th ,, . . .	6·68	5·02	5·83	4·45	Milky stage.
22nd ,, . . .	6·69	5·37	4·94	4·82	More of starch.

TABLE IV.

pH values of soil, root and leaf of rice plants manured with the mixture of ammonium sulphate and sodium nitrate at different stages of growth.

Date 1932	pH of the soil	pH of the root	pH of the leaf	pH of the inflorescence	Remark
5th July . . .	6·91	..	..	..	Before transplantation.
2nd August . . .	6·41	6·16	5·82	..	After transplantation.
26th ,, . . .	7·02	4·43	6·13	..	After manuring.
1st September . .	5·98	4·43	6·07	..	No rains.
12th ,, . .	6·45	6·97	5·82	..	Rains.
16th ,, . .	6·97	7·37	5·77	..	,,
25th ,, . .	6·31	6·76	5·65	..	
10th October . . .	6·81	4·78	6·16	6·41	
15th ,, . . .	6·64	4·92	6·00	5·97	Raining.
19th ,, . . .	6·54	4·63	5·67	4·56	Starchy.

On studying these results it will be seen that in the unmanured soil the pH value continues to fall up to the middle of August thus showing an increase in acidity (Table I). Then there is a rise in pH value with a fall in the second week of September, again the soil becoming acidic. Again a rise in pH follows during the remaining period. The results show that the soil becomes acidic and neutral alternately twice during the whole season (Fig. 1).

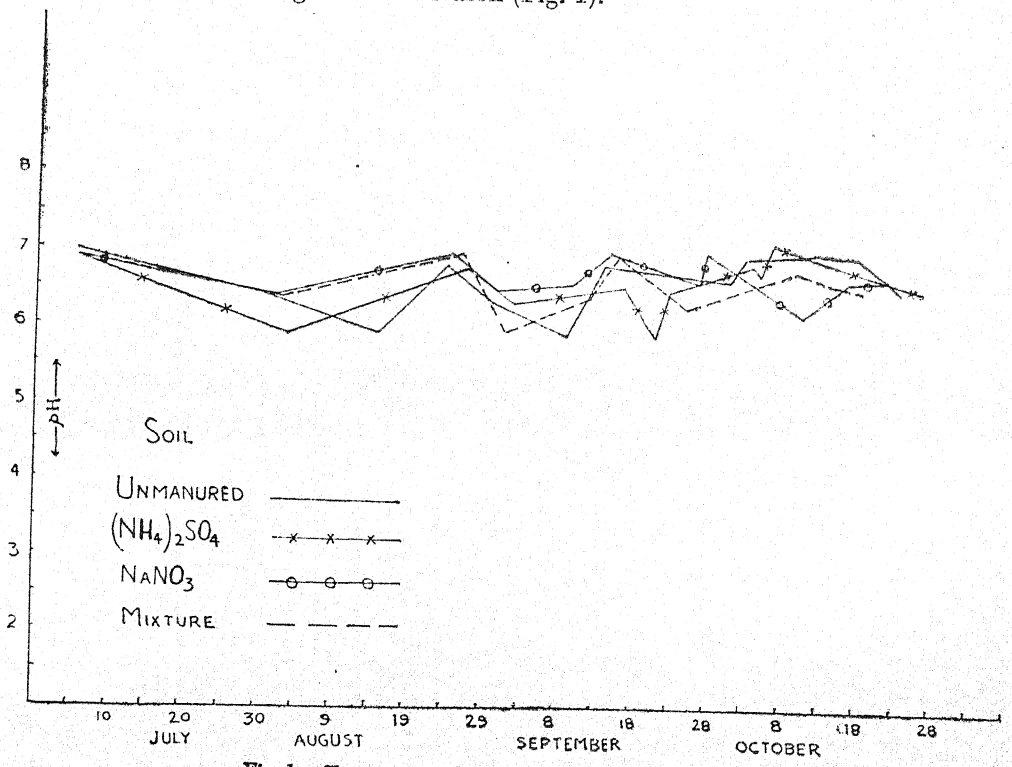


Fig. 1. Changes in the pH values at different periods.

The soil manured with sodium nitrate shows a rise in the pH value instead of a fall indicating that the soil becomes more alkaline and it remains more alkaline than the unmanured soil uptil October (Table III).

The soil manured with sulphate of ammonia (Table II) passes through the same sequence of changes of hydrogen ion concentration as the unmanured soil.

In the case of the soil manured with the mixture of ammonium sulphate and sodium nitrate (Table IV) the fall in pH value is correspondingly less than those in the pH values of the unmanured soil or soil manured with ammonium sulphate. This may be due to the presence of sodium nitrate. After some time the fluctuations in the pH value of the soil manured with the mixture correspond with the fluctuations in the pH value of the unmanured soil.

As it is well known that plants manured with ammonium sulphate grow better than plants manured with sodium nitrate, it appears that the rice plants grow better in acidic soils and the acidity of the soil is probably produced on account of the absorption of ammonium ions as shown by Dastur and Malkani [1933] during the early stages of growth.

The changes in pH values of the roots of the rice plants in the unmanured and the manured soil are similar though different in magnitudes (Tables I and IV). The roots become more acidic from August till the 1st week of September and the pH value of the roots begins to rise after which there is a decline in the pH value (Fig. 2).

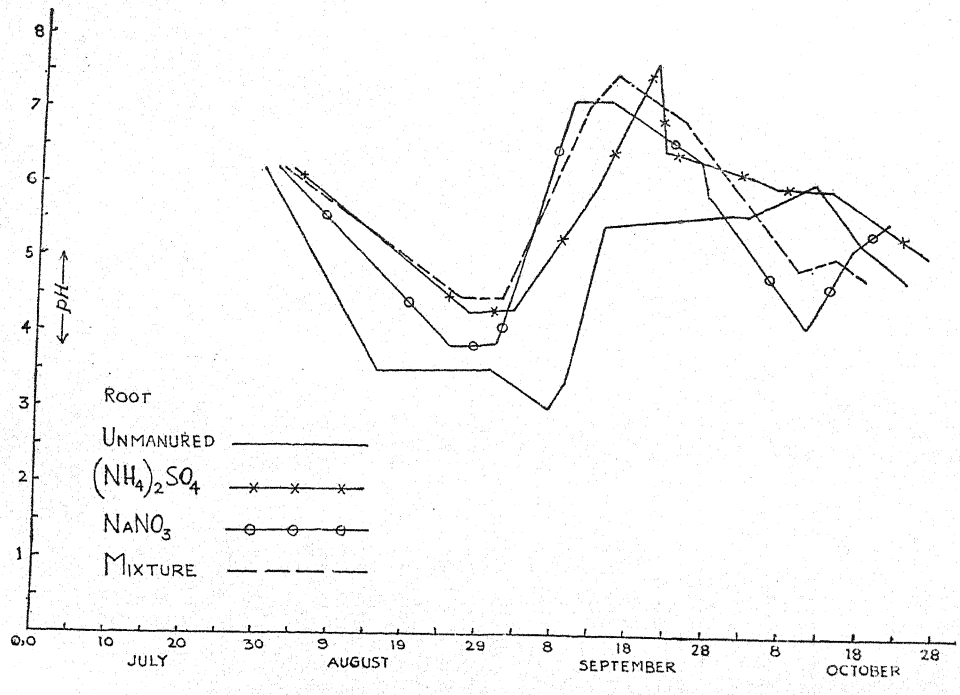


Fig. 2. pH values of the roots of rice plants.

In the case of the roots of unmanured plants the acidity of the roots reaches its highest point of pH 2·94 on the 7th September (Table I). In the case of the roots of the plants manured with ammonium sulphate the roots show lesser acidity the highest point being pH 4·23 (Table II). In the case of the roots of plants manured with the mixture the acidity is still less (Table IV).

In the middle of September the pH value of the unmanured roots shows a rise of about pH 2·0. This rise is common in the roots of all plants manured with different nitrogen containing salts. In plants manured with sodium nitrate pH of the roots rises from 3·8 to 7·01 ; in plants manured with ammonium sulphate it rises from 4·23 to 7·52 and in plants manured with the mixture it rises from pH 4·43 to 7·37. In the case of manured plants the second fall in the pH value of roots starts earlier than in the case of unmanured plants (Fig. 2). In the case of the mixture the acidity of the roots is greater than that of the roots of plants manured with either nitrate or ammonium sulphate in the 1st half of October.

In the last week of October the roots reach the value of pH 4·6 and this may be due to the death of tissues, when the proteins reach their iso-electric point as the protoplasm undergoes heterosis due to ageing. It will be interesting to compare this pH value of the proteins obtained when the tissues are dead with the pH value of the iso-electric point obtained by direct measurement in the third part of this investigation. The pH values of the inflorescence confirm the point that the approach of iso-electric point causes the ageing of the tissues. Just when the inflorescences were opened, their pH values were of the same values as those of the leaves.

Inflorescence pH	6·03	6·23	6·41	6·41
Leaf pH	6·05	6·05	6·08	6·16

As the ripening of the seeds went on, the pH values decreased so much so that when the seeds became hard the ρH values of the inflorescence were 4·45 which is about the pH of the iso-electric point.

The results of the changes in pH values of the leaves of the unmanured rice plants indicate that the pH value of the leaves remains more or less uniform (Fig. 3). The changes in pH values are rather smaller fluctuating between pH 5·9 and 6·2.

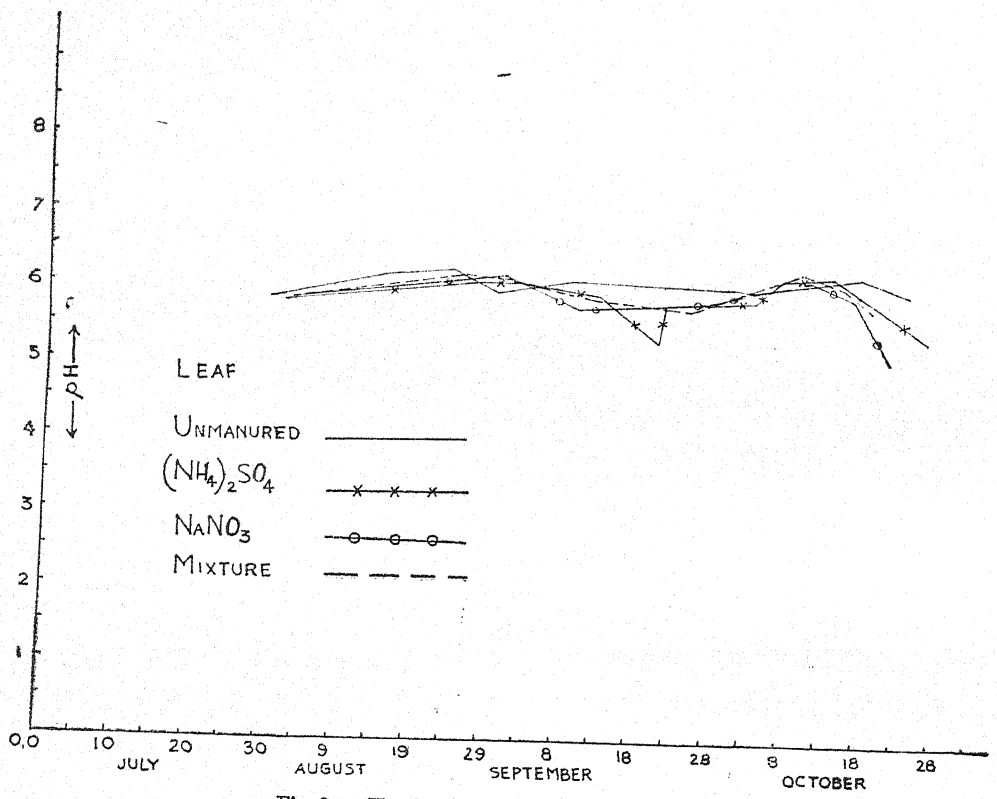


Fig. 3. pH values of the leaves of rice plant.

In the case of the plants manured with ammonium sulphate the fluctuations in the pH values of the leaves are slightly greater than in the unmanured plants. The most significant point is the increased acidity of the leaves when the roots show the increased alkalinity (Figs. 2 and 3). The pH of the leaf falls from 6·02 to 5·25 in September when the pH of the roots rises from 4·23 to 7·52. The same is the case in the leaves and roots of plants manured with sodium nitrate and ammonium sulphate. When there is a rise in pH value of the roots there is a corresponding, though a slight, fall in the pH value of the leaves. The pH values of the leaves of the rice plant, in all the four cases show very small fluctuations, the graphs showing the changes in the pH values of the leaves from the unmanured and manured beds, are nearly straight lines.

It was also considered important to study the changes in the pH values of the soil, root and leaf during the day. So the measurements of the hydrogen ion concentration of the soil and plant tissues are made every six hours during the

day from the manured and unmanured beds. The samples are taken early in the morning, at noon, evening, and midnight. The results of these determinations are given in Tables V-VIII. Though the fluctuations in the pH values are not regular, still there is clear indication that pH values of roots and soil begin to rise from morning to evening and then begin to fall till next morning. The leaves show quite an irregular behaviour. Mason and Maskell [1929] in their studies on the transport of nitrogenous substances in the cotton plants and diurnal variations, observe that the nitrogenous content of the leaf increases by day and diminishes by night. Ingalls and Shive [1931] in their study on the relation of hydrogen ion concentration of tissue fluids to the distribution of iron in plants show that hydrogen ion concentration of tissue fluids varies with light intensity, low hydrogen ion concentration (high pH) corresponds to high light intensity and high hydrogen ion concentration (low pH) corresponds to low light intensity, and the soluble iron content also fluctuates and is directly proportional to the hydrogen ion concentration of respective fluids. But whatever may be the factor influencing, with the results obtained here no general conclusion can be drawn.

TABLE V.

Diurnal changes in the pH values of the soil, root and leaf of rice plants unmanured.

Date	Time	pH value of the soil	pH value of the root	pH value of the leaf
1932				
1st October	7 a.m.	6·66	5·50	5·97
" "	1 p.m.	6·76	5·82	5·92
" "	7 p.m.	6·79	5·92	6·10
2nd "	1 a.m.	7·06	6·45	5·92
3rd "	7 a.m.	6·97	5·49	5·92
" "	1 p.m.	7·12	6·31	6·25
24th "	7 a.m.	6·51	4·59	5·87
" "	1 p.m.	6·66	5·17	5·64
" "	7 p.m.	6·86	4·71	5·40
25th "	1 a.m.	7·40	4·15	5·87

TABLE VI.

Diurnal changes in the pH values of the soil, root and leaf of rice plants manured with ammonium sulphate.

Date	Time	pH value of the soil	pH value of the root	pH value of the leaf
1932				
21st September	7 a.m.	5·92	7·52	5·25
" "	1 p.m.	6·74	6·97	5·64
22nd "	7 a.m.	6·21	6·35	5·73
" "	1 p.m.	6·54	7 21	5·67
" "	7 p.m.	7·22	6·84	5·56
23rd "	1 a.m.	6·58	6·45	5·60
" "	7 a.m.	6·53	6·33	5·72
6th October	7 p.m.	6·43	5·92	5·98
7th "	1 a.m.	6·33	5·92	5·67
" "	7 a.m.	7·16	5·85	6·05
" "	1 p.m.	6·88	5·62	6·26

TABLE VII.

Diurnal changes in the pH values of the soil, root and leaf of rice plants manured with sodium nitrate.

Date	Time	pH value of the soil	pH value of the root	pH value of the leaf
1932				
10th September	7 a.m.	6·63	7·01	5·69
" "	1 p.m.	6·97	7·24	5·17
27th "	7 a.m.	6·66	6·18	5·77
" "	1 p.m.	6·74	6·35	5·85
" "	7 p.m.	6·84	5·98	5·92
28th "	1 a.m.	6·35	6·64	5·88
21st October	1 p.m.	6·38	4·97	5·73
" "	7 p.m.	6·58	5·92	5·70
22nd "	1 a.m.	7·19	4·63	5·27
" "	7 a.m.	6·69	5·37	4·94

TABLE VIII.

Diurnal changes in the pH values of soil, root and leaf of rice plants manured with mixture of ammonium sulphate and sodium nitrate.

Date	Time	pH value of the soil	pH value of the root	pH value of the leaf
1932				
24th September	1 p.m.	6·84	7·87	5·72
" "	7 p.m.	6·56	6·99	5·47
25th "	1 a.m.	6·68	6·33	5·67
" "	7 a.m.	6·31	6·76	5·65
19th October	7 a.m.	6·54	4·63	5·67
" "	1 p.m.	6·88	5·70	5·19
" "	7 p.m.	6·99	5·82	5·60
" "	1 a.m.	6·18	5·92	5·59

II. The determination of hydrogen-ion concentration of the solutions of sodium nitrate, ammonium sulphate and the mixture of the two salts, and of the seedlings kept in them.

It is established by Dastur and Malkani [1933] that rice seedlings absorb the two ions of ammonium sulphate unequally, the ammonium ion absorbed being greater than the sulphate ion. If that is the case the solution of ammonium sulphate should become acidic. In order to confirm the above results it was undertaken to study the changes in the pH values of the roots and leaves of the rice seedlings before and after they had remained in the ammonium sulphate solution of different concentrations, and to determine their reaction on the external solutions. It is also considered necessary to repeat these observations with the solution of sodium nitrate and of the mixture of sodium nitrate and ammonium sulphate.

The data thus obtained would help also in the interpretation of results obtained in section III of this investigation on the iso-electric point of the tissues of rice plant.

Experimental.

Rice grains are soaked in water for twenty-four hours and allowed to germinate in saw dust. Watering was done twice a day, once in the morning and once in the evening. When they are seven days old they are taken out of the saw dust and washed thoroughly with tap water. The stronger seedlings of equal size were selected and the seeds were removed carefully from the seedlings. The solutions of ammonium sulphate, sodium nitrate and of the mixture of them separately are taken in rectangular jars of about 1200-c. c. capacity and in each jar 90 seedlings are kept to grow. The jars are fitted with wooden covers bearing six holes, each hole carrying 15 seedlings. The inside of the jars was completely paraffined so as to prevent any change in the pH value of the solutions due to solubility of glass in water, and the outside was blackened with Japan black. The covers were cemented with paraffin to the jars. Three culture jars are set up for each experiment having a total number of 270 seedlings for each strength of the solution. The strengths of the solutions of sodium nitrate, ammonium sulphate and a mixture of the two used are $N/100$, $N/200$, $N/300$, $N/400$, $N/500$. It was found that some of the seedlings did not survive for seven days in some of the solutions according to their concentrations. The solutions were aerated every morning through a hole in the centre of the wooden cover.

The pH value of each solution is determined before the seedlings are kept. The extraction of the sap of roots and leaves of rice seedlings is made and the pH values are determined before the seedlings are kept in the solution. The same process is repeated after the seedlings had remained in each solution for seven days. The sap of the roots and leaves is extracted according to the method already described.

The main difficulty that arose in the experiments was in the determination of the pH value of the ammonium sulphate solution. Successive readings of the same ammonium sulphate solution by the same electrode were not constant. The second reading was always lower than what it really ought to be. Many determinations were made and it was later found out that the electrodes were poisoned on account of the adsorption of free ammonia on the surface of the electrodes and therefore the saturation of the hydrogen-ion concentration on the electrode was not complete. This difficulty was overcome by treating the electrodes kept in distilled water with hydrogen for half an hour after each determination of the pH value of ammonium sulphate solution so that free ammonia absorbed was reabsorbed by distilled water.

TABLE IX.

pH values of the roots and leaves of rice seedlings (seven days old) and of the external solutions of ammonium sulphate.

Date	Description	pH of the solution of $(NH_4)_2SO_4$	pH of the roots	pH of the leaves	No. of seedlings alive out of 270
	N/100				
8th February 1933	Before	5·25	4·09	4·58	
15th ,, ,,	After	5·06	5·25	4·92	132
	N/200				
9th ,, ,,	Before	5·09	4·09	4·58	
16th ,, ,,	After	4·41	4·69	4·97	128
	N/300				
10th ,, ,,	Before	4·76	3·93	4·53	
17th ,, ,,	After	3·52	3·27	4·88	211
	N/400				
11th ,, ,,	Before	4·63	3·93	4·53	
18th ,, ,,	After	3·19	3·36	5·09	218
	N/500				
28th March ,,	Before	4·20	4·09	4·53	
4th April ,,	After	2·78	4·00	5·06	196

TABLE X.

pH values of the roots and leaves of rice seedlings (seven days old) and of the external solutions of sodium nitrate.

Date	Description	pH of the solution of $NaNO_3$	pH of the roots	pH of the leaves	No. of seedlings alive out of 270
	N/100				
16th January 1933	Before	8·51	4·64	4·68	
23rd ,, ,,	After	8·45	5·02	5·29	80
	N/200				
25th ,, ,,	Before	8·07	4·51	5·09	
1st February ,,	After	8·07	5·82	6·18	80

TABLE X—*contd.*

Date	Description	pH of the solution of $NaNO_3$	pH of the roots	pH of the leaves	No. of seedlings alive out of 270
	N/300				
25th January 1933 .	Before . .	7·70	4·51	5·09	
1st February „ .	After . .	6·94	5·92	6·21	85
	N/400				
30th January „ .	Before . .	7·40	4·83	4·59	
6th February „ .	After . .	6·48	5·21	6·08	86
	N/500				
16th January „ .	Before . .	6·98	4·64	4·68	
23rd „ „ .	After . .	5·92	4·79	5·82	110

TABLE XI.

pH values of the roots, and leaves of rice seedlings (seven days old) and of the external solutions of the mixture of ammonium sulphate and sodium nitrate.

Date	Description	pH of the solution of mixture	pH of the roots	pH of the leaves	No. of seedlings alive out of 270
	N/100				
27th February 1933 .	Before . .	7·07	4·09	4·43	
6th March „ .	After . .	7·07	5·31	5·29	7
	N/200				
28th February „ .	Before . .	6·88	4·09	4·58	
7th March „ .	After . .	6·88	4·89	5·30	179
	N/300				
12th „ „ .	Before . .	6·41	4·09	4·45	
19th „ „ .	After . .	6·44	4·86	5·47	184
	N/400				
16th „ „ .	Before . .	6·18	4·09	4·53	
23rd „ „ .	After . .	6·02	4·54	5·49	247
	N/500				
22nd „ „ .	Before . .	5·85	4·09	4·53	
29th „ „ .	After . .	5·59	4·83	5·44	224

The results in Table IX show that the ammonium sulphate solution becomes more and more acidic as the concentration diminishes. The pH value of $N/100$ ammonium sulphate is pH 5·25 while that of $N/500$ ammonium sulphate is 4·20.

In all the concentrations of ammonium sulphate the external solution becomes markedly acidic after the seedlings had remained in them for seven days. The results clearly support the findings of Dastur and Malkani [1933] that the ammonium ion is absorbed in greater proportion than the sulphate ions. It can also be seen that the differences between pH values of the external solutions of ammonium sulphate before and after the seedlings had remained in them become greater as the concentration of ammonium sulphate solution decreases. With $N/100$ ammonium sulphate the difference is pH 0·19 while with $N/500$ ammonium sulphate the difference between before and after becomes pH 1·42 (Table IX). These results also confirm the findings of Dastur and Malkani [1933] that the percentage of ammonium ion absorbed from ammonium sulphate solution becomes greater and greater as the concentration decreases. The pH value of the roots increases after they are kept in $N/100$ and $N/200$ ammonium sulphate solutions while in lower strengths of the ammonium sulphate solution there is a decrease in the pH value of the roots. It is also noticed that the death rate of the rice seedlings kept in $N/100$ and $N/200$ ammonium sulphate solutions is higher than in ammonium sulphate solutions of lower strengths. In $N/100$ ammonium sulphate solution 132 remained alive while in $N/200$, 128, in $N/300$ solution 211 remain alive and in $N/400$ ammonium sulphate solution 218 were alive (Table IX).

The leaves in all cases show an increase in the pH value after the seedlings are treated with ammonium sulphate solutions. The increase in pH value is greater in $N/400$ and $N/500$ solutions than in the more concentrated ones (Table IX).

The pH values of sodium nitrate solutions of different strengths (Table X) are much higher than those of the corresponding ammonium sulphate solutions. The sodium nitrate solutions are markedly alkaline in higher concentrations while they are rather acidic in the lower concentrations. There is not much difference in the pH values of the $N/100$ and $N/200$ sodium nitrate solutions before and after the seedlings are kept in them. In lower strengths of sodium nitrate there is a decrease in pH value of the solutions indicating the absorption of sodium ions. The pH value of the roots rises in $N/100$ to $N/400$ sodium nitrate solutions. In $N/200$ and $N/300$ sodium nitrate solutions there is a rise of pH 1·4 in the pH values of the roots while in the lower strengths the rise in pH becomes less and less. The death rate of the seedlings in the first four strengths of sodium nitrate is very high—about 80 seedlings out of 270 survived—while in $N/500$ the number of seedlings surviving

is 110. The results indicate that the rice plants in order to continue their normal activity should probably have an acidic medium.

In the case of the mixture of two salts (Table XI) the pH value of the solutions does not show any change after the seedlings had remained in them for seven days in the first three concentrations, while in $N/400$ and $N/500$ solutions there is a small lowering of the pH value.

In the case of sodium nitrate solutions and the mixture the pH value of the roots is always lower than the final pH values of the external solutions while the reverse is the case in ammonium sulphate solutions. The ammonium sulphate solution is rendered more acidic by the greater absorption of ammonium ions while the sodium nitrate solution is more alkaline than the roots on account of the lesser absorption of sodium ions and greater basic properties of sodium ions. In the case of mixture the external solution is more alkaline than the roots on account of the presence of sodium ions through the degree of alkalinity as compared to the pH of the roots is less in the mixtures than in the sodium nitrate solutions.

In all the above experiments the pH values of the external solutions are determined after a period of seven days. It would be interesting to study the changes in the hydrogen-ion concentration of the ammonium sulphate solution every day after the rice seedlings had remained in it. Side by side the hydrogen-ion concentration of the leaves and the roots must be determined in order to study the corresponding changes occuring in them. From the results given in Table IX it will be seen that the greatest change in the pH of the ammonium sulphate solutions used occurred in $N/400$ and $N/500$ solutions after the rice seedlings remained in them for a week. It was therefore undertaken to study the changes in the pH value of $N/500$ ammonium sulphate solution and of the leaves and roots of the rice seedlings kept in it for every twenty-four hours for seven days. It was necessary to prepare seven sets of culture jars with $N/500$ ammonium sulphate solution and 270 rice seedlings kept in each set as before. The pH values of the external solution and the leaves and roots are determined before keeping the seedlings in the solutions. The pH values of the ammonium sulphate solution and of the leaves and roots of the rice seedlings of one set of jars were determined every day. Thus the daily changes in the pH values of the ammonium sulphate solution and of the leaves and the roots were determined for a week. The daily changes in the pH are given in Table XII.

The pH of the external solution of ammonium sulphate is 4·20. It shows a rise for the first two days and is 4·46 at the end of the second day. Then there is a continuous fall in the pH values of the ammonium sulphate solution. The

total fall in the pH value on each day from the beginning and the daily fall in pH on each day are given in the following tables.

TABLE XII.

The determinations of pH values of root and leaf of rice seedlings (seven days old) and of ammonium sulphate N/500 before and after keeping for different number of days in the week.

Date	Description	pH of the amonium sulphate N/500	pH of the root	pH of the leaf	Number of seedlings alive out of 270
	1 day				
15th May 1933 . .	Before . .	4·20	4·10	4·53	
16th ,, ,, . .	After . .	4·30	3·51	4·68	270
	2 days				
10th ,, ,, . .	Before . .	4·20	4·10	4·53	
12th ,, ,, . .	After . .	4·46	3·87	5·24	268
	3 days				
6th ,, ,, . .	Before . .	4·20	4·10	4·53	
9th ,, ,, . .	After . .	4·10	2·94	4·78	232
	4 days				
6th ,, ,, . .	Before . .	4·20	4·10	4·53	
10th ,, ,, . .	After . .	3·31	2·83	4·79	227
	5 days				
10th ,, ,, . .	Before . .	4·20	4·10	4·53	
15th ,, ...	After . .	2·98	3·24	4·86	212
	6 days				
5th ,, ,, . .	Before . .	4·20	4·10	4·53	
12th ,, ,, . .	After . .	2·84	3·62	4·93	208
	7 days				
28th March 1933 . .	Before . .	4·20	4·10	4·53	
5th April ,, . .	After . .	2·78	4·00	5·06	196

TABLE XIII.

The daily changes in the pH value of the N/500 solution of ammonium sulphate with the rice seedlings (seven days old).

Period	Total fall in the pH	Period	Daily fall in the pH
1st—2nd day . . .	—0·10	1st—2nd day . . .	—0·10
1st—3rd ,, . . .	—0·26	2nd—3rd ,, . . .	—0·16
1st—4th ,, . . .	+0·10	3rd—4th ,, . . .	+0·36
1st—5th ,, . . .	+0·89	4th—5th ,, . . .	+0·79
1st—6th ,, . . .	+1·22	5th—6th ,, . . .	+0·33
1st—7th ,, . . .	+1·36	6th—7th ,, . . .	+0·14
1st—8th ,, . . .	+1·42	7th—8th ,, . . .	+0·06

There is a total fall of pH 1·42 on the 8th day (after seven days). The daily fall in the pH of the solution of ammonium sulphate shows a maximum between the 4th and 5th day after which the fall in the pH decreases. On the 7th day there is a very slight fall in the pH value. Dastur and Malkani [1933] have shown that the absorption of ammonium ion and the sulphate ceases on the 8th day and they have shown that the external solution of ammonium sulphate reaches its equilibrium with the internal concentration of the same salt; then further absorption ceases. These results are confirmed by the results of the daily changes in the pH values of the external solution. Further decrease in the pH value of the ammonium sulphate solution ceases as the unequal absorption of the ammonium and sulphate ions ceases. The fall in the pH value of the ammonium sulphate clearly shows that the ammonium ions are absorbed in larger amounts than the sulphate ions (Fig. 4).

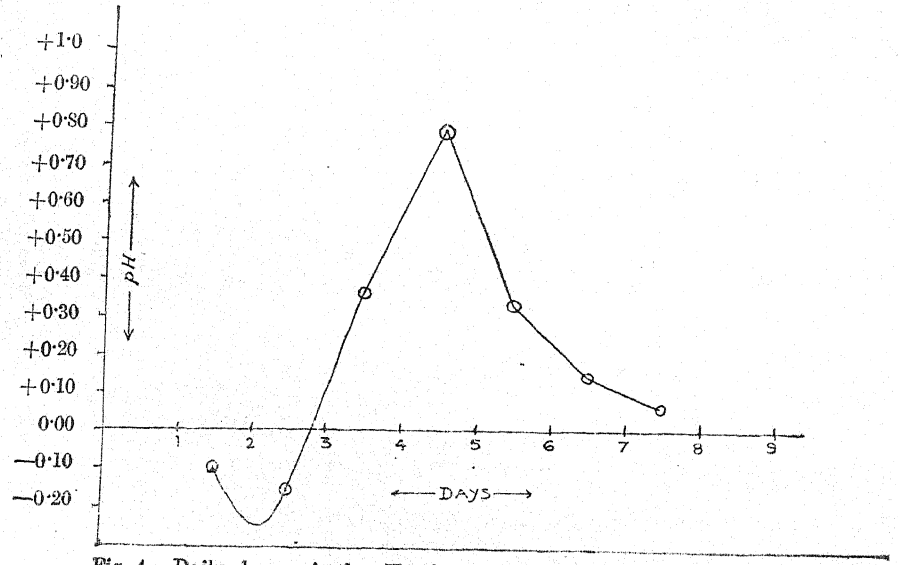


Fig. 4. Daily changes in the pH value of the ammonium sulphate solution.

The pH value of the sap of the roots is 4·10 when placed in the ammonium sulphate solution. The pH value shows a fall and it reaches its lowest point on the 4th day. The pH value of the roots then begins to rise and on the 7th day it is again 4·0, very nearly equal to its original value before they were placed in the ammonium sulphate solution. These changes in the pH values of the roots are of the same type as those found in the rice plants grown in the soil. The roots become acidic first and then again they lose in acidity. In the ammonium sulphate solution the same type of changes are noticed. On the 7th day when the equilibrium with the external solution is reached the roots regain their original pH value.

The pH values of the leaves of the rice seedlings are higher than those of the sap of the roots and they pass through the same type of changes as the roots. On the seventh day the pH of the sap of the leaves shows increase on the original pH of the sap of the leaves.

III. The determinations of iso-electric points of the tissues of rice plant.

The conception of the iso-electric point was originally evolved by Hardy for solutions of certain artificially prepared pure substances; but this conception can not be lightly applied without modifications to the heterogeneous mixture of plasmatic colloidal system. The cells and the tissues of some degree of acidity may exhibit characteristics which correspond with the behaviour of pure ampholytes in the zone near their iso-electric point. Our knowledge of the iso-electric point of cells and tissues has been largely extended by Robbins. According to the hypothesis of protoplasmic hysteresis, the approach of iso-electric point is the cause of the ageing of the tissues. By the term 'protoplasmic hysteresis' is meant a continuous condensation assisted by the changing of colloids into a less dispersed state, in which the adsorption of water decreases in consequence of a diminution of the adsorbing surfaces. This hypothesis of protoplasmic hysteresis does not harmonise with the conception of Pearsall and Priestley [1923]. According to Pearsall and Priestley [1923] the plant meristem requires to be relatively free from water in order to persist in its synthetic metabolism. The vacuolated cells bordering upon the meristem withdraw water from meristem, by their osmotic action; principal proteins in meristematic tissues should have iso-electric point at reactions lying between pH 3 and 6. The establishment of any relation between protein behaviour *in vitro* and the behaviour of the protoplasmic membrane seemed therefore to be a matter of great importance.

Pearsall and Ewing [1924] developed a method to determine the protoplasmic iso-electric point directly. The basis of the experiments is the comparison of the rates of diffusion of some easily determined ion from living tissues kept in solutions

of different hydrogen-ion concentrations, and the chloride ion is deemed convenient for this purpose. Equal volumes of the tissues of the rice plants were placed in a series of conical flasks containing 100 c.c. of hydrochloric acid solutions of varying hydrogen-ion concentrations for definite period. The chlorine ion is measured argentometrically with the aid of potassium chromate. Increase or decrease of chlorine is found by comparison with measurements on solutions from which tissues are absent. The acid series of solutions were neutralised with dilute sodium hydrate before the chlorine was estimated and three chlorine estimations on 20 c. c. of liquid were carried out on each solution. The results obtained with the roots of 50 rice seedlings are given in Table (XIV). They show the movements of chlorine ion to or from the roots in hydrochloric acid. The pH values of the hydrochloric acid solutions used are also given.

Similarly the movements of chlorine ions to and from the leaves of 50 rice seedlings in hydrochloric acid are given in Table XV. The graphs showing the gain and loss of chlorine ions from the roots and leaves of the rice seedlings at various hydrogen-ion concentration are given in Fig. 5. According to these results of the diffusion of chlorine ions the iso-electric point of the protoplasm of the leaves lies between pH 4·1 and 4·25 and that of the roots between pH 4·30 and pH 4·40.

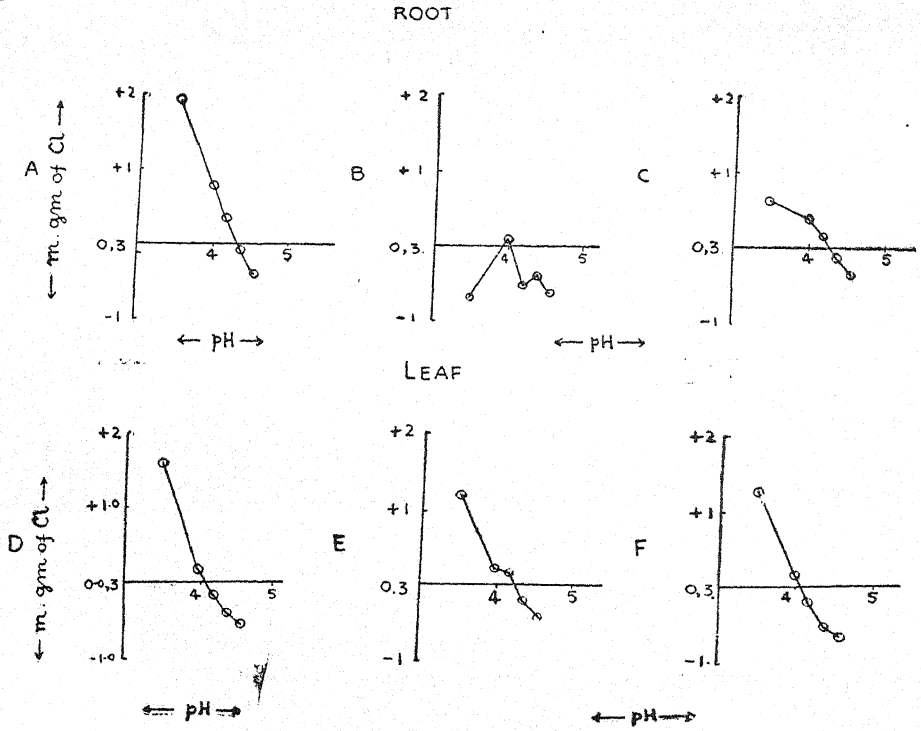


Fig. 5. Gain and loss of chlorine ions from the roots and leaves of rice seedlings.

According to these determinations of the iso-electric point of the chief proteins of protoplasm the latter may be on the acidic side of its iso-electric point if the hydrogen-ion concentration of the sap is less than 4·30 in the case of the roots and 4·1 in the case of the leaves. The proteins of protoplasm are on the alkaline side of the iso-electric point if the hydrogen-ion concentration of the cell sap is greater than 4·4 and 4·25 in the roots and leaves respectively. The determinations of iso-electric points of the proteins of the roots are important as they throw light on the permeability of ammonium and nitrate ions at different stages of growth of the rice plant.

TABLE XIV.

Diffusion of chlorine ions to and from 50 roots of rice seedlings, and the iso-electric point of the roots.

Volume of $N/100$ HCl	pH of the HCl solution	Loss or gain of chlorine ions from tissues in mgs.		
		I	II	III
c. c.				
7·2	3·5	+1·920	+0·70	+0·64
3·9	4·0	+0·780	+0·11	+0·39
2·9	4·2	+0·355	—0·53	+0·19
2·0	4·4	—0·070	—0·43	—0·14
1·2	4·6	—0·390	—0·64	—0·35
	Iso-electric point	4·35	4·4	4·3

TABLE XV.

Diffusion of chlorine ions to and from 50 leaves of rice seedlings, and the iso-electric point of the leaves.

Volume of $N/100$ HCl	pH of the HCl solution	Loss or gain of chlorine ions from tissues in mgs.		
		I	II	III
c. c.				
7·2	3·5	+1·60	+1·21	+1·28
3·9	4·0	+0·18	+0·24	+0·18
2·9	4·2	—0·18	+0·17	—0·18
2·0	4·4	—0·43	—0·21	—0·49
* 1·2	4·6	—0·56	—0·43	—0·64
	Iso-electric point	4·10	4·25	4·10

Conclusions.

The determinations of the pH values of the roots of the rice plants show two important features. The graph given in Fig. 3 shows four bimodal curves. The pH value of the roots first rises from June to July from pH 4·09 to 6·18 and then declines from July to August and reaches its lowest point on or about 31st August. Afterwards, the pH value steadily rises and reaches its second maximum in the 3rd week of September and then again falls. This indicates that the roots of the rice plants are acidic in reaction, become more alkaline and then reach their highest point of acidity at the end of August. The roots then gradually become less and less acidic till they are more or less neutral or alkaline in reaction.

These changes in the hydrogen-ion concentration of the roots can be correlated with the characteristic behaviour of the roots in the absorption of ammoniacal and nitrate ions. It is shown by Dastur and Malkani [1933] that ammonium and sulphate ions from ammonium sulphate are absorbed equally from $N/200$ and $N/300$ ammonium sulphate solutions but in the lower concentration of the ammonium sulphate solutions the percentage of ammonium ions absorbed becomes greater and greater than that of sulphate ions absorbed as the concentration of ammonium sulphate solution is decreased. They have also shown that the nitrate ions are very little absorbed during the early stages and its absorption gradually increases from July to September. These characteristic absorptions of ammonium, sulphate and nitrate ions can be adequately explained on the results obtained from the hydrogen-ion concentration of the roots and the iso-electric points of the proteins of the rice plants.

It is shown by Bigwood [1930] that the permeability of the gels to electrolytes is chiefly controlled by two factors, one simple physical diffusion and second chemical reaction between the penetrating ions and the electrolytes present in the gel. The penetration of ammonium ions in the protoplasm of the roots is first controlled by the diffusion factor. In this case the diffusion of ammonium ions is a case of selective absorption and this selective absorption of ammonium ions occurs on account of the following reasons.

The iso-electric points of the proteins of the roots of the rice plants as determined in section III of the investigation are in the zone of 4·30—4·4. The pH value of the roots of the rice plants during the 1st month of its growth rises from 4·09 to 6·18; the proteins of the protoplasm of the roots will be on the alkaline side of the iso-electric point. So the proteins of the roots will combine more readily with cations or basic ions than with anions or acidic ions. Since ammonium ions carry the positive charge, they are absorbed more readily than the sulphate or nitrate ions which carry the negative charge. The results obtained by Dastur and

Malkani [1933] show that the nitrate ions from nitrates are absorbed in very minute quantities and sulphate ions from ammonium sulphate are absorbed to a considerable extent. If the ionic charge is responsible for the absorption of these ions, then both nitrate and sulphate ions should be absorbed nearly in equal quantities from these salts as both are acidic ions. It is also shown by Dastur and Malkani [1933] that the percentage of nitrate ions absorbed by the seedlings 15 and 30 days old is greatest from the solution of ammonium nitrate and is least from the solution of sodium nitrate. The order for decreasing absorption of nitrate ions from different salts is ammonium nitrate, magnesium nitrate, calcium nitrate, potassium nitrate, sodium nitrate. It can be argued that this differential absorption of nitrate is favoured by the greater absorption of ammonium ions from ammonium nitrate on account of the following reasons.

The pH value of the ammonium nitrate solutions is very near the pH values of the iso-electric point of the proteins of the roots, while the pH values of the sodium nitrate solutions are very far removed on the alkaline side, from the pH of the iso-electric point of the proteins. So in the case of ammonium nitrate solution, the protoplasm takes up basic ammonium ions and consequently the nitrate ions to certain extent. (When it is stated here that the basic ion is absorbed or the acid ion is not absorbed, it means that the one is absorbed in greater proportions than the other.) In the same way from ammonium sulphate solution when ammonium ions are absorbed there will be certain amount of absorption of the sulphate ions. But in the case of sodium nitrate, and potassium nitrate the basic potassium or sodium ions are absorbed to such small extent on account of their stronger basic properties that the negatively charged ions are still less absorbed. So there are two reasons for the non-absorption of nitrate ions from potassium nitrate or sodium nitrate solutions. The proteins of the protoplasm of the roots are on the alkaline side of their iso-electric point so the proteins will combine more readily with basic ions or cations than with the acidic ions or anions. Secondly the potassium or sodium ions are absorbed to a very small extent on account of the markedly basic properties of sodium and potassium ions. As these basic ions are absorbed to a small extent the nitrate ions are absorbed still to a lesser extent.

When the ammonium ions are absorbed by the rice seedlings there is an increase in the hydrogen-ion concentration of the cell sap of the roots. This can be seen from the results obtained with the rice seedlings kept in ammonium sulphate solutions (Tables IX and XII) when the acidity of the cell sap of the roots increases after the seedlings had been kept for a week in the ammonium sulphate solutions of lower concentrations.

The cell sap of the roots of the rice plants grown in soil becomes more and more acidic from July to August and the acidity produced may be due to the second factor controlling the permeability of gels to electrolytes. The ammonium ions absorbed will not remain as such in the roots but they naturally react with the electrolytes in the protoplasm to produce indiffusible substances [Northrop, 1929]. The proteins of the protoplasm probably dissociate into more of COOH groups and this dissociation of COOH ions brings about the acidity of the roots [Pearsall and Priestley, 1923]. The proteins of the roots are now placed in an acidic medium and are on the acidic side of the iso-electric point and consequently the anions or acidic radicals are taken up in greater proportion by the roots than the cations or basic ions. So the nitrate ions will continue to be absorbed during the latter stages of growth. Secondly the solutions of potassium nitrate and sodium nitrate have their pH values far away from the iso-electric point on the alkaline side. Consequently the absorption of acidic ions increases. Then nitrate ions absorbed will have to be neutralised and therefore the dissociation of the NH_2 groups of the proteins occurs which ultimately brings about the alkalinity of the cell sap of the roots.

It is thus clearly seen why the rice plants are able to absorb ammoniacal nitrogen in the earlier stages of growth and nitrate nitrogen in the later stages of growth. During the earlier stages of growth the pH of the proteins of the protoplasm is on the alkaline side of their iso-electric point and the roots are also in an alkaline medium. This favours the absorption of the basic ions. As the ammonium ions have low basic properties they are absorbed in greater amounts than potassium or sodium ions. The nitrate nitrogen is not taken in as the nitrate ions are acidic and the proteins combine less readily with the anions than with the cation. The non-absorption of the nitrate ions is greater as the potassium and sodium ions are still less absorbed. From ammonium nitrate solutions the nitrate ions are absorbed to a great extent on account of the greater absorption of the ammonium ions. The same holds good for the sulphate ions from the ammonium sulphate solutions.

During the later stages of growth the pH of the proteins comes on the acidic side of the iso-electric point and therefore the acidic ions like the nitrate ions are more and more absorbed as the age advances and the absorption of ammonium ions decreases. Thus the diffusion of ammonium and nitrate ions into the roots of the rice plants depends upon the pH of the proteins of protoplasm. The changes in the pH brings about the preferential absorption of one or the other ion as the case may be.

The pH value of the roots becomes 4·5 at the end of October. This value is very near the value of the pH of the proteins at their iso-electric point. It clearly

shows that when the roots die the proteins reach their iso-electric point on account of the protoplasmic hysteresis. The pH value of the iso-electric point of the proteins as determined directly is nearly the same. The same holds good for the pH of the inflorescence. The pH value of the inflorescence is 6·03, 6·23, 6·41, 6·41 during the first stages of growth. But when the grains ripen and become hard the pH value of the inflorescence decreases and reaches the value of 4·45 which is near the pH value of the proteins at their iso-electric points.

Summary.

It is shown by Dastur and Malkani [1933] in their study on the intake of nitrogen by rice plants that there is unequal absorption of ammonium and sulphate ions from the solutions of ammonium sulphate and the same holds good for all ammonium salts. Secondly, the absorption of ammonium ions decreases as the plant ages, while the absorption of nitrate ions from the nitrates, though very little in the early stages, increases in the later stages of growth.

It is shown that the abosorption of ammoniacal nitrogen in the early stages and the nitrate ions in the later stages is correlated to the iso-electric point of the proteins of the protoplasm of the tissues of the rice plant.

The investigation is divided into three parts.

(1) The determinations of the changes in the pH value of the soil and of the rice plants treated with ammonium sulphate, sodium nitrate and a mixture of the two salts on equal nitrogen basis and of untreated rice plants.

(2) The determinations of the pH values of the solutions of the above salts before and after the rice seedlings, seven days old, had remained in them for a fixed period and the determinations of the pH values of the rice plants before and after they had remained in the solutions.

(3) The third part deals with the determinations of the iso-electric point of the proteins in the protoplasm of the roots and leaves of the rice seedlings.

The iso-electric point of the rice tissues is determined by the method of measurement of the rates of diffusion of the chlorine ions from the living tissues kept at different hydrogen-ion concentrations.

The results show that in all cases the pH value of roots of the rice plants first rises and then begins to fall in August, reaches its minimum point in the last week of August and then rises to the maximum value in the middle of September after which there is a gradual fall in pH values.

In the case of the leaves pH value begins to rise in August up to the end of that month, then begins to fall and reaches their minimum in the middle of September

and begins to rise till they reach their second maximum point in the second week of October after which there is a fall.

It is seen that the points of minima in the case of the roots coincide with those of the maxima of the leaves. The pH value of the roots undergoes a wider range of fluctuations while the pH of the leaves shows small fluctuations probably owing to the presence of strong buffer substances in the sap of the leaves.

The pH value of the soil decreases up to the end of July and then rises up to the end of August and again falls in the first week of September. It then rises in the middle of September and then smaller fluctuations follow for a week and then begins the fall.

The pH values of the soil manured with sodium nitrate stand always the highest. The pH values of the soil manured with ammonium sulphate stand lowest in the pH scale, while the pH of the soil manured with the mixture stands midway between the two. These relations also hold good for the culture solutions.

The results show that the iso-electric point of the plant tissue lies between pH 4·1 and 4·4. At this hydrogen-ion concentration the proteins of the protoplasm will neither behave as acids nor as bases, as the electric charge on the proteins is neutral.

The results show that the pH values of the roots lie on the alkaline side of the iso-electric point of their proteins and therefore the latter will combine more readily with the basic ions than with the acidic ions. As the ammoniacal nitrogen is in the form of the basic ion it is absorbed in the earlier stages of growth while the nitrate ions being acidic are very little absorbed. The reverse is the case during the later stages of growth. The pH of the roots is on the acidic side of the iso-electric point and therefore the proteins of the roots absorb acidic ions in preference to basic ions. Therefore the absorption of nitrate nitrogen takes place while the absorption of the ammoniacal nitrogen diminishes.

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THE BIOLOGY OF *HOLCOCERA PULVEREA* MEYR. (BLASTOBASIDÆ), ITS PREDATORS, PARASITES, AND CONTROL.

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(With Plate LV and five text figures)

INTRODUCTION.

The moth *Holcocera pulverea* is one of the most injurious insects found associated with lac in India. Its larvæ are predatory on the lac insect, *Laccifer (Tachardia) lacca*, and cause considerable damage to the lac crops. Meyrick [1907] described the moth and placed it under the genus *Blastobasis*. In 1908, however, he transferred it from the genus *Blastobasis* to *Hypatima* Hb. Lord Walsingham and Durrant [1909] found the genus *Hypatima* HS. (nec. Hb.) to be a synonym of *Holcocera* Clemens. Stebbing [1910] says that specimens of *Hypatima pulverea* were obtained by Stevens in Kumaun and bred by Troup at Dehra Dun from *kusum* (*Schleichera trijuga*) lac received from Raipur, C. P. Imms and Chatterjee [1915] say that *Hypatima pulverea* is even more abundant than *Eublemma amabilis*, and inspite of its smaller size is almost equally destructive. They found it in large numbers in all the lac they received from various forests except Hoshangabad (C. P.); from eleven separate consignments of lac from this area they could breed only six moths. They also remark that these moths are most abundant from April to November and scarce from December to March, and that the larvæ of this moth are extremely destructive to stick lac, and do not appear to be dependent upon living lac growing on trees. Fletcher [1920] records this moth from *rahar* (*Cajanus indicus*) lac, Kumaun; *ber* (*Zizyphus Jujuba*) lac, Pusa;

palas (*Butea frondosa*) lac, Berar, C. P., *kusum* (*S. trijuga*) lac, Palamau; lac from Bengal, and from "Galls" Coimbatore. He also gives a short account of the larva and pupa. Mahdihassan [1925] collected the moth from material received from various localities in India. Misra [1929] has briefly dealt with it in his revised bulletin. On the basis of the work being carried out in the Department, Glover [1930] enumerates some of its enemies and gives a short preliminary note on its bionomics. He also records it from various localities in Burma from the material collected by Mrs. Norris [1931], the Director of the Institute. We have been dealing with the insect for the last six years and the results obtained during our study are discussed in the following pages.

Methods used.

To study the prevalence and the seasonal history of the predator and its enemies, lac crops grown at Namkum were examined weekly and samples caged monthly in parasite boxes from the immature to mature stage. Distribution was worked out from the samples received from different parts of India and Burma. Experiments on longevity and oviposition were carried out in battery jars of 4in.×3in. to 6½in. × 4½in. dimensions. The jars were covered with perforated paper or brass wire wove covers and the moths fed on sugar solution. Oviposition cages were provided with lac sticks free from the eggs of the predator. The eggs deposited on these sticks were daily removed into small glass capsules for further development and hatching. The newly hatched larvæ were next transferred to lac sticks from which all stages of the predator had been removed. Fresh lac sticks of the aforesaid type were provided to the growing larvæ when they had devoured the lac insects and the lac on the previous one, till they pupated. In the dry summer months of May and June oviposition was also tried in moist cages, and was found more frequent in them than in the dry ones.

Egg.

The freshly laid egg is colourless and translucent; with the growth of the embryo the colour of the egg passes through light pink to deep pink. When the embryo is fully developed, the larva can be seen moving in the transparent egg-shell. The egg is more or less oval in shape, but slightly narrower towards the micropylar end (Fig. 1*a*). The exochorion is ornamented with concentric closely situated small papillate prominences. The circles increase in diameter from either end towards the middle of the egg. The micropyle is situated at the narrow end on an elevated area and is clearly visible. The egg is 0·5 mm. in length and 0·3 to 0·33 mm. in breadth.

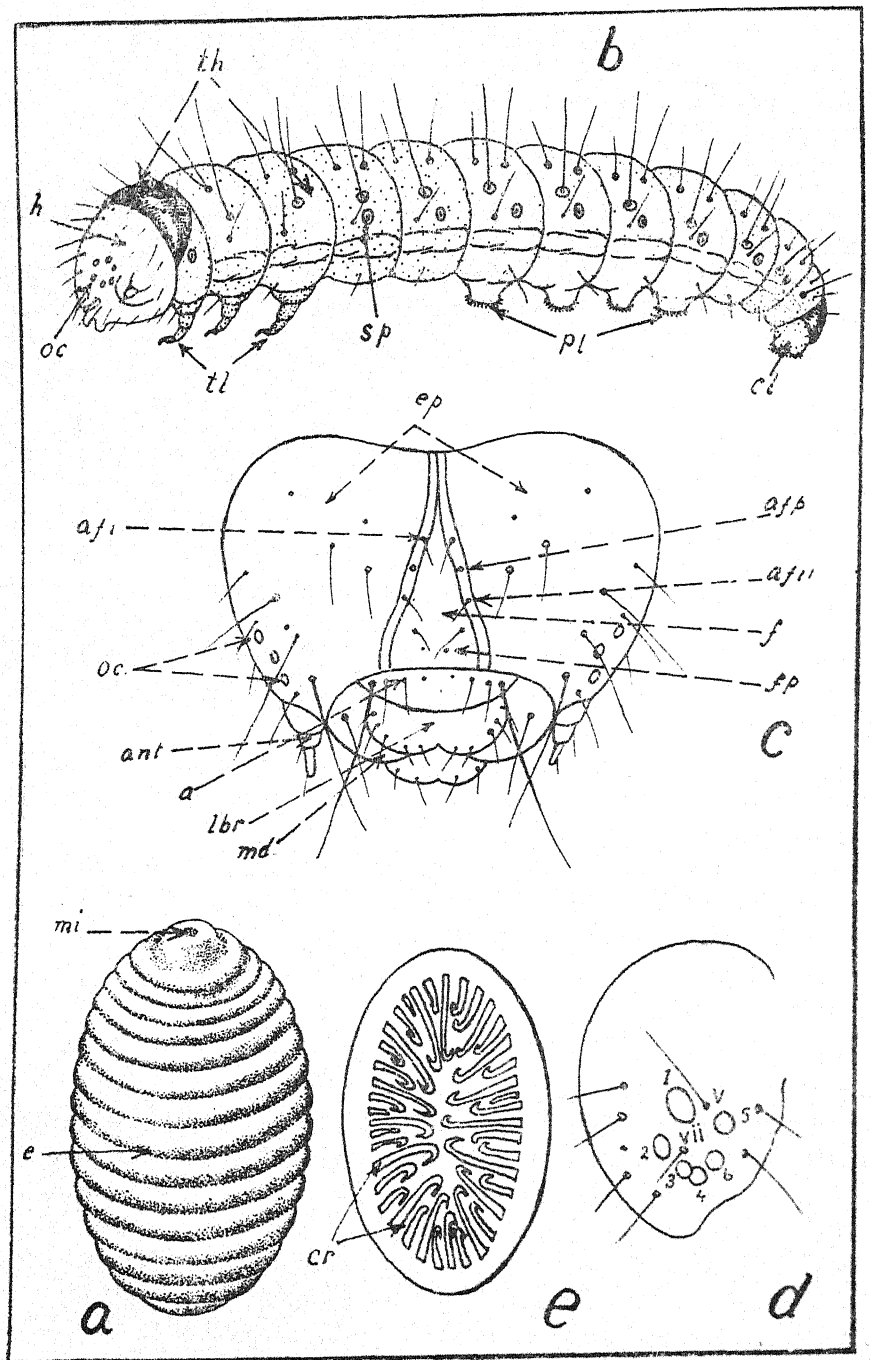


Fig. 1. (*a*). Freshly laid egg (×106). (*b*). Mature larva (×11). (*c*). Head of mature larva (×35). (*d*). Arrangement of ocelli and neighbouring setae (×35). (*e*). Crochets (×150).

(*For key to lettering see page* 864.)

The unfertilised egg differs from the fertilised one in having a dull white colour with greenish tinge, and shrivels up without hatching.

The minimum and the maximum length of the egg stage and the figures for average egg stage (Table V) show that the duration of egg stage rises from November to January (in generation 2 and 3) and begins to drop from February (in generation 3) and remains more or less at its lowest from April to October (in generation 4, 5 and 1). The minimum duration of hatching (the period within which all the eggs laid on the same day complete hatching) throughout the year is one day and the maximum 6 days in December. The highest period in other months varies from 2 to 3 days.

LARVA.

The newly emerged larva is about 1·38 mm. in length. Dorsally it has a shining appearance ; the head is chocolate coloured. prothorax light brown. and the rest of the body pale yellow.

The mature larva (Fig. 1 *b*) measures 10-12 mm. in length and 2 mm. in breadth. The body is cylindrical, slightly tapering posteriorly, dirty brown in colour, head bilobed, brown, with fairly long dark setæ, labrum and region immediately about it dark grey to deep brown. Frons (Fig. 1 *c*) inverted Y-shaped, brown interiorly but black or dark grey on edges. The ocelli (Fig. 1 *d*) are six in number ; third, fourth and sixth situated close together. Prothoracic shield dark with a narrow median stripe ; thoracic legs ringed with black, three-jointed and ending in a claw ; spiracles nine pairs, oval, brown, ringed with black, one on the prothorax and the remaining eight pairs on the first eight abdominal segments. The metathorax bears a pair of rudimentary spiracles. The tracheal tube can be seen arising from it. Each of the abdominal segments third to sixth and the tenth bears a pair of single lobed fleshy prolegs. Each proleg has a circular arrangement of triordinal crochets (Fig. 1 *e*) ; the number of crochets in each leg is variable. The last abdominal segment is chitinised and brown dorsally.

Larval instars.

It was first believed that the larval stage of each generation had nine instars. But later studies revealed that the number of instars in the larval stage of the moth varied in the two main lac crops (*Katki* * and *Baisakhi* †). It was then that in each month of the year the number of times the larva moulted and the width of the head capsule of the larva after each moult were studied. The results obtained from this study are recorded in Tables I and II.

* Lac crop progeny of broods other than *kusum* infected in June-July, matures in October-November.

† Lac crop progeny of broods other than *kusum* infected in October-November matures in June-July.

The data in the tables show that (1) the July to September larvæ which do not hibernate have five instars in each generation and probably the same happens with the nonhibernating larvæ of October. (2) The October larvæ which hibernate have in each generation nine instars and probably the same is the case with the hibernating larvæ of September. (3) The November to April larvæ and perhaps of May and June too have nine instars. It is difficult to explain the sudden change from five to nine instars in the larval stage of the two seasons; but possibly the comparative high humidity from July to October allows the integument of the larva with the least periodical sheddings to accommodate itself more easily to the growth of the larva than the low humidity from November to June and, therefore, the larval stage of each generation has five instars from July to October (in the *Katki* crop) and nine instars from November to June (in the *Baisakhi* crop).

The tables fully support Dyar's law [1915] and also the statement that if the measurements of a certain instar are missed, the average ratios of increase are greatly affected and, therefore, the calculated measurements do not approximate closely to the actual measurements in all cases. Such cases in the tables are marked with an asterisk.

TABLE I.

Larval instars. width of head capsule in mm.

Months	Type of measurement	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	Average ratio of increase
July	Act.	0·200	0·280	0·440	0·640	0·880	1·450
	Calc.	0·200	0·290	0·420	0·609	0·883	
	Act.	0·200	0·200	0·300	0·500	0·900	1·450
	Calc.	0·200	0·290	0·420	0·609	0·883	
	Act.	0·200	0·200	0·300	0·640	0·900	1·460
	Calc.	0·200	0·292	0·426	0·621	0·906	
	Act.	0·200	0·200		0·640	0·900	1·350*
	Calc.	0·200	0·270	0·364	0·491	0·662	
	Act.	0·200	0·200	0·400	0·620	0·900	1·450
	Calc.	0·200	0·290	0·420	0·609	0·883	
	Act.	0·200	0·280	0·400	0·560	0·900	1·450
	Calc.	0·200	0·290	0·420	0·609	0·883	

*Instar was missed. See text for explanation.

TABLE I—*contd.*

Months	Type of measurement	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	Average ratio of increase
July	Act.	0·200	0·280	0·360	0·640	0·900	1·460
	Calc.	0·200	0·292	0·426	0·621	0·906	
	Act.	0·200	0·280	0·360	0·660	0·900	1·460
	Calc.	0·200	0·292	0·426	0·621	0·906	
August	Act.	0·200	0·280	0·360	0·500	0·902	1·460
	Calc.	0·200	0·292	0·426	0·621	0·906	
	Act.	0·200	0·260	0·420	0·580	0·900	1·460
	Calc.	0·200	0·292	0·426	0·621	0·906	
	Act.	0·200	0·260	0·420	0·580	0·900	1·460
	Calc.	0·200	0·292	0·426	0·621	0·906	
	Act.	0·200	0·260	0·420	0·580	0·900	1·460
	Calc.	0·200	0·292	0·426	0·621	0·906	
	Act.	0·200	0·260	0·400	0·540	0·800	1·410
	Calc.	0·200	0·280	0·397	0·559	0·788	
	Act.	0·200	0·280	0·380	0·680	0·902	1·460
	Calc.	0·200	0·292	0·426	0·621	0·906	
September	Act.	0·200	0·300	0·500	0·700	0·960	1·480
	Calc.	0·200	0·296	0·438	0·648	0·959	
	Act.	0·190	0·300	0·400	0·600	0·800	1·435
	Calc.	0·190	0·273	0·392	0·559	0·792	
	Act.	0·190	0·300	0·400	0·640	0·840	1·456
	Calc.	0·190	0·277	0·403	0·587	0·855	
	Act.	0·200	0·280	0·400	0·600	0·700	1·375
	Calc.	0·200	0·275	0·378	0·519	0·713	

TABLE II

Larval instars, width of head capsule in mm.

Months	Type of measurement	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	6th Instar	7th Instar	8th Instar	9th Instar	Average ratio of increase
October	Act.	0·200	0·280	0·360	0·543	0·700	0·740	0·900	1·040	1·120	1·250
	Calc.	0·200	0·250	0·313	0·391	0·489	0·611	0·764	0·955	1·193	
	Act.	0·200	0·280	0·400	0·600		0·800	0·900	1·040	1·120	1·270*
	Calc.	0·200	0·254	0·323	0·410	0·520	0·660	0·836	1·061	1 347	
	Act.	0·190	0·280	0·400	0·600	0·700	0·800	0·900	1·000	1·120	1·260
	Calc.	0·190	0·239	0·361	0·379	0·478	0·602	0·759	0·956	1·204	
November	Act.	0·200	0·250	0·300	0·380	0·480	0·600	0·760	0·980	1·140	1·240
	Calc.	0·200	0·248	0·307	0·380	0·471	0·584	0·724	0·897	1·112	
	Act.	0·200	0·260	0·320	0·380	0·460	0·600	0·750	0·960	1·120	1·240
	Calc.	0·200	0·248	0·307	0·380	0·471	0·584	0·724	0·897	1·112	
	Act.	0·200	0·250	0·300	0·360	0·480	0·600	0·760	0·980	1·120	1·240
	Calc.	0·200	0·248	0·307	0·380	0·471	0·584	0·724	0·897	1·112	
December	Act.	0·200	0·260	0·300	0·360	0·480	0·600		0·960	1·120	1·230*
	Calc.	0·200	0·246	0·302	0·371	0·456	0·560	0·688	0·846	1·040	
	Act.	0·200	0·240	0·300		0·500	0·600	0·760	0·980	1·120	1·220*
	Calc.	0·200	0·244	0·297	0·362	0·441	0·538	0·656	0·800	0·976	
January	Act.	0·190	0·240	0·300	0·420	0·540	0·600	0·700	0·840	0·940	1·220
	Calc.	0·190	0·231	0·282	0·344	0·420	0·512	0·625	0·763	0·930	
	Act.	0·190	0·240	0·320	0.500	0·600	0·700	0·800	0·920	1·000	1·240
	Calc.	0·190	0·236	0·293	0·363	0·450	0·558	0·692	0·858	1·063	
February	Act.	0·200	0·250	0·300	0·360	0·480	0·620	0·760	0·960	1·100	1·230
	Calc.	0·200	0·246	0·302	0·371	0·456	0·560	0·688	0·846	1·046	

*Instars were missed. See text for explanation.

TABLE II—*contd.*

Months	Type of measure-ment	1st Instar	2nd Instar	3rd Instar	4th Instar	5th Instar	6th Instar	7th Instar	8th Instar	9th Instar	Average ratio of increase
March	Act.	0·190	0·240	0·300	0·360	0·500	0·640	0·800	0·900	1·000	1·240
	Calc.	0·190	0·236	0·293	0·363	0·450	0·558	0·692	0·858	1·063	
	Act.	0·190	0·240	0·300	0·360	0·440	0·500	0·600	0·700	0·900	1·220
	Calc.	0·190	0·231	0·282	0·344	0·420	0·512	0·625	0·763	0·930	
	Act.	0·190	0·240	0·300	0·340	0·440	0·600	0·700	0·800	0·900	1·220
	Calc.	0·190	0·231	0·282	0·344	0·420	0·512	0·625	0·763	0·930	
	Act.	0·190	0·240	0·306	0·400	0·500	0·600	0·700	0·800	0·900	1·220
	Calc.	0·190	0·231	0·282	0·344	0·420	0·512	0·625	0·763	0·930	
	Act.	0·190	0·260	0·300	0·360	0·480	0·600	0·740	0·800	0·920	1·220
	Calc.	0·190	0·231	0·282	0·344	0·420	0·512	0·625	0·763	0·930	
	Act.	0·190	0·260	0·300	0·380	0·480	0·600	0·700	0·800	0·900	1·226
	Calc.	0·190	0·231	0·282	0·344	0·420	0·512	0·625	0·763	0·930	
April	Act.	0·200		0·320	0·400	0·490	0·640	0·760	0·840	0·960	1·200*
	Calc.	0·200	0·240	0·288	0·345	0·414	0·496	0·595	0·714	0·856	
	Act.	0·200	0·250		0·370	0·460	0·660	0·740	0·900	0·960	1·210*
	Calc.	0·200	0·242	0·292	0·353	0·427	0·516	0·624	0·755	0·913	
May											
June											

* Instars were missed. See text for explanation.

Larval habits.

The larva comes out of the egg by biting a longitudinal slit near the micropylar end. It generally enters the lac incrustation by biting a hole at the base of the lac, but sometimes it may also enter through either the anal tubercular orifice or the spiracular openings of the test. After this, it goes on tunnelling and feeding on

the lac insects and their tests from below leaving behind the bits of destroyed incrustation and its own excreta interwoven in a thin, loose but tough, web of silken threads. It also feeds on the dead lac insects and the resin alone. The excreta of the early larval instars is white and granular and that of the later instars forms dark crimson lumps, which are surcharged with lac, and the dark crimson colour is due to the undigested lac dye.

The larva, unlike that of the predator *Eublemma amabilis* which after hatching enters straight into the lac incrustation, and devours the lac insect and its test completely before proceeding further, has often been found to bite holes into the tests of several neighbouring lac cells before it enters one and while tunnelling the incrustation leaves some of the lac insects and their tests half eaten. It thus causes more damage to the lac crop than would seem to be necessary for its food while the *Eublemma amabilis* larva damages the crop only to the extent it is needed for its maintenance. An individual caterpillar, during the larval life is capable of devouriug 7 to 45 matuie lac females or about 64 to 449 immature lac cells.

The full-fed mature larva becomes sluggish and begins to spin a white cocoon in its gallery (Fig. 2*a*). The cocoon is made of several layers of fine white silk except at the head end where it is thinner. The cocoon is tough and strong, and one cannot easily remove the larva uninjured from it unless it is carefully opened either with a pair of fine scissors or a sharp scalpel. The larva pupates inside the cocoon after an inactive state of about two days during the warmer months and ten days during the colder months.

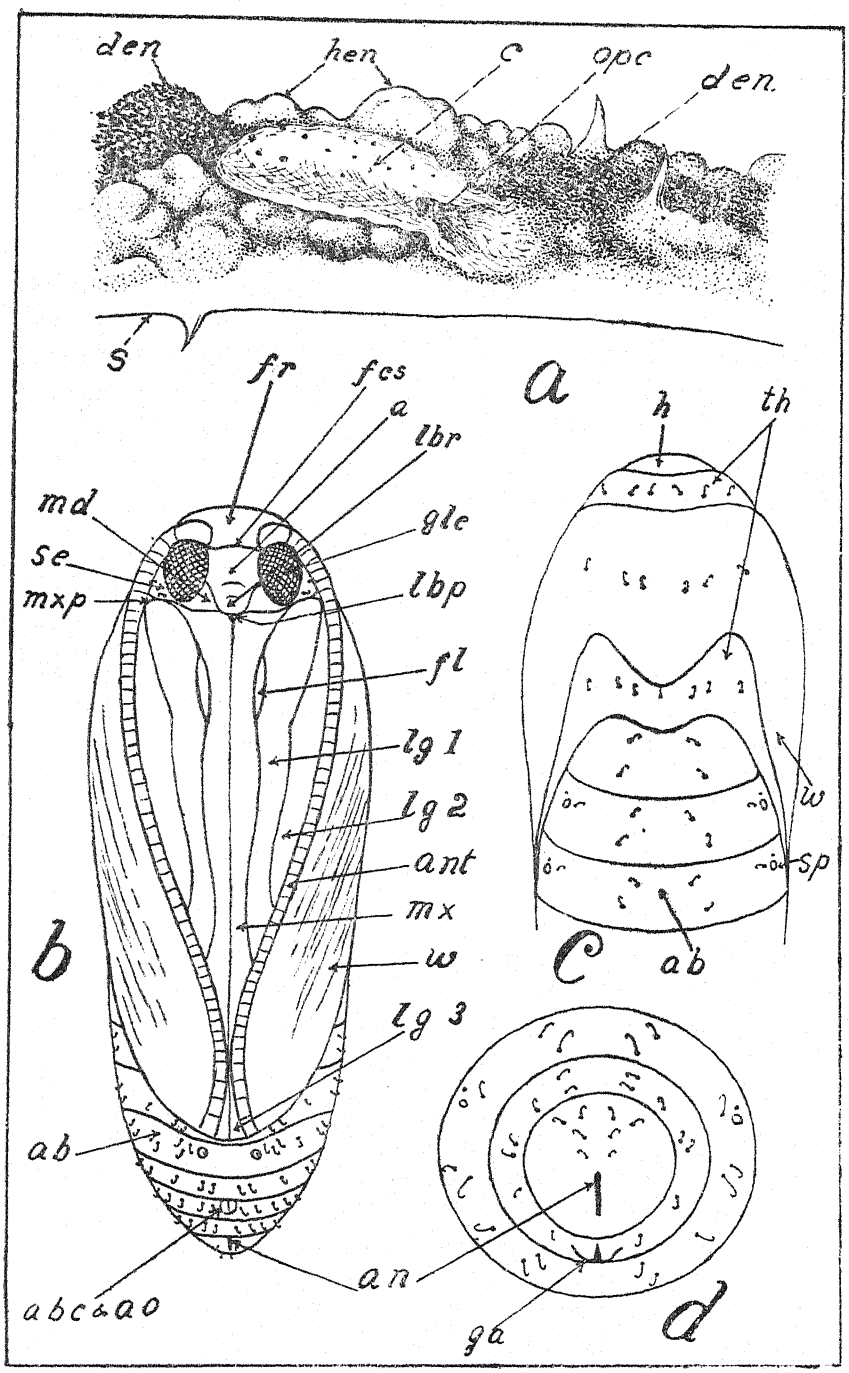


Fig. 2. (*a*). Lac stick showing damage done by the predator, and its cocoon (×2·5). (*b*). Female pupa ventral view (×17·5). (*c*). Female pupa dorsal view of thorax and first three abdominal segments (×17·5). (*d*). Male pupa last three abdominal segments (×43·75).

(For key to lettering see page 864.)

The length of the larval stage (Table V) also varies according to the seasons of the year and more or less follows the same rule as the egg stage.

Pupa.

The pupa (Fig. 2 *b-c*) of either sex is at first, light brown in colour, but with maturity the colour changes to deep brown except in the last two or three segments which remain slightly lighter in colour. Excluding the head, there are three thoracic and ten abdominal segments. Mesothorax is the largest, and none of the abdominal segments is movable. The pupa has minute recurved hooks on its body, they are entangled in the silken cocoon. The cremaster is absent. Fletcher [1920] mentions six cremastral hooks on the pupa; but we do not find any other armature on the anal segment except the eight recurved hooks similar to those on other segments of the body (Fig. 2*d*.) Each of the second to eighth abdominal segments bears a pair of spiracles. In the female pupa the opening of the oviduct and bursa-copulatrix (Fig. 2*b*) is situated on the 8th sternum, but in the male the genital opening (Fig. 2*d*) lies at the junction of the eighth and ninth sterna approximating more towards the ninth than the eighth sternum. The anus lies ventrally on the tenth segment. The male and female pupae are respectively 4·5 and 5·5 mm. in length and 1·5 and 1·75 mm. in width (average of ten of each sex). The monthly average pupal period varied from 7·3 to 13·5 days in male and 7·5 to 13 in female. The maximum and minimum periods for both were 18 days in October and 5 days in July respectively. The adult emerges by breaking the fronto-clypial portion of the pupal coat and the thinly woven portion of the enclosing cocoon near its head (Fig. 2*a*.)

Adult.

Description of the male and female (Plate LV, figs. 1 and 2).—Since Meyricks's original description of the male and female moth is given under the two separate species, viz., *H. deleropa* and *H. pulverea*, and also in view of a few minor differences met with by us it has been deemed advisable to redescribe the moth keeping Meyricks's description as far as possible intact.

Head ochreous-whitish, crown irrorated with fuscous. Palpi whitish irrorated with dark grey or with fuscous except apex of the second joint, terminal joint in male slender, acute. Antennae in female pale greyish-ochreous and in male pale greyish-ochreous to dark grey and hairy. Thorax ochreous whitish, irrorated with fuscous. Abdomen in female pale greyish-ochreous, bulged out in middle in male grey-whitish mixed with grey, segments with ferruginous bands. Fore wings elongate, rather narrow, moderately pointed, apex tolerably obtuse, greyish-whitish, suffusedly irrorated with dark fuscous; discal stigmata fairly distinct in female and cloudy in male, round, dark fuscous at one-half the distance in male and three-fourths in female, cilia pale greyish sprinkled with whitish points and with a few

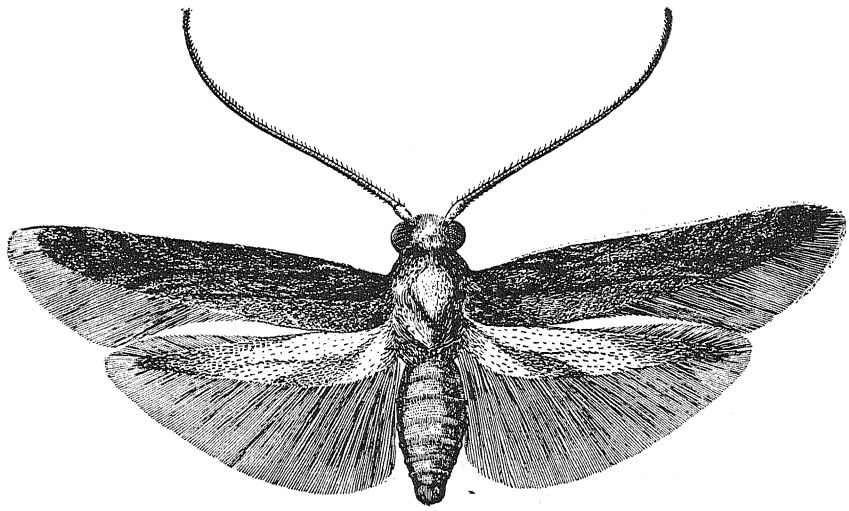


Fig. 1. *Holcocera pulverea* ♂ (×7½).

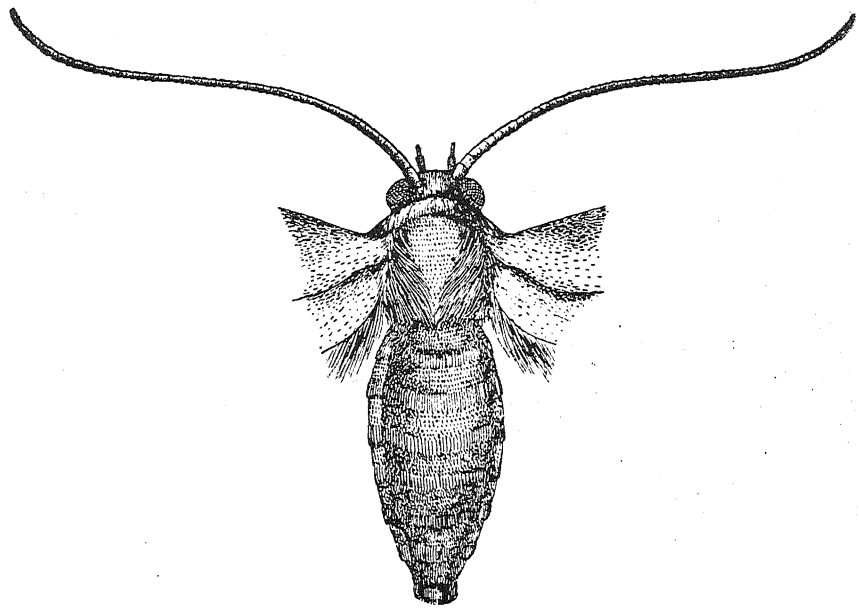


Fig. 2. *Holcocera pulverea* ♀ (×10½).



dark fuscous scales towards base. Hind wings with vein *media* connate with or without stalk of radius grey, towards base and in cell pale and subhyaline, cilia light grey, slightly ochreous-tinged. Wing expanse in female (average of 15) 14 mm. in male (average of 15) 13·1 mm. Length of body in female 5·63 mm. (average of 15) and in male 5·0 mm. (average of 15). When the moth is at rest forewing completely overlaps the posterior.

The venation of the fore and hind wings show some variations in media. In the fore wing out of 23 specimens examined 21 corresponded to Fig. 3*a* and of the remaining two in one specimen M_1 and M_2 after arising from the discal cell converge to join each other about the middle of their course and afterwards run separately (Fig. 3*b*). In the other M_2 and M_3 after arising separately from the discal cell run coalesced to some distance and then separate again; this specimen also shows both (cu1a and cu1b) (Fig. 3*c*). In the hind wing out of the 26 cases examined 10 corresponded to Fig. 3*d* and 9 to Fig. 3*e*; in the latter M_2 and M_3 separate soon after a common origin from the discal cell while in the former they do so after running as one joint vein for some distance. In the remaining seven cases M_2 and M_3 were found to arise separately from the discal cell and in five of them (Fig. 3*f*) they were also found to run separately throughout their course but in two M_2 instead of running its whole course separately was found to converge to meet M_3 as shown in Fig. 3*g*.

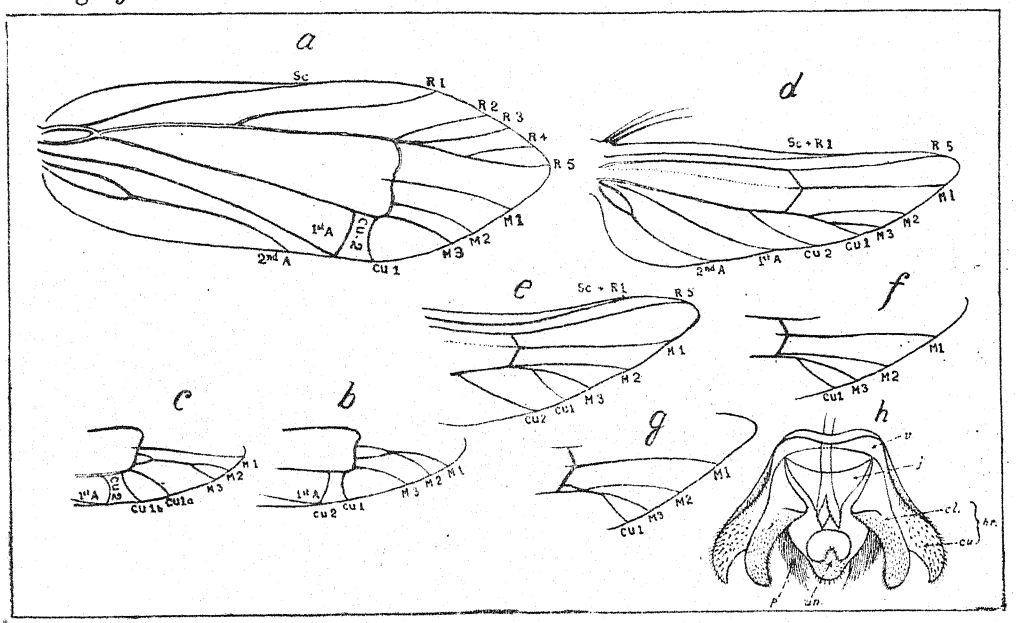


Fig. 3. (*a*). Venation of fore-wing (×10).
(*b* & *c*). Variations in media of fore-wing (×10).
(*d*). Venation of hind wing (×10).
(*e*, *f* & *g*). Variations in media of hind wing (×10).
(*h*). Male external genitalia (×30).

(*For key to lettering see page* 864.)

Prominent external differences between the female and male moth.

——	Female	Male
1. Antennae . . .	Covered with small hairs and therefore do not look hairy, 45 to 48 jointed.	Hairy, 40 to 30 jointed.
2. Size and colour .	Bigger than the male and colour also darker.	Smaller than female, colour lighter.
3. Abdomen . . .	Broader in the middle	Narrower, gradually tapers towards the posterior end.
4. Last abdominal segment viewed posteriorly.	A complete circular ring of scales through which the ovipositor may or may not be seen.	A complete circular ring of scales within which the peniculus of each side looks like an arc. (Fig. 3*h*.)

Longevity.

To find out the duration of the adult stage in each month of the year moths of either sex were confined in battery jars, and the results obtained are summarized in Table III and the averages plotted in Fig. 4.

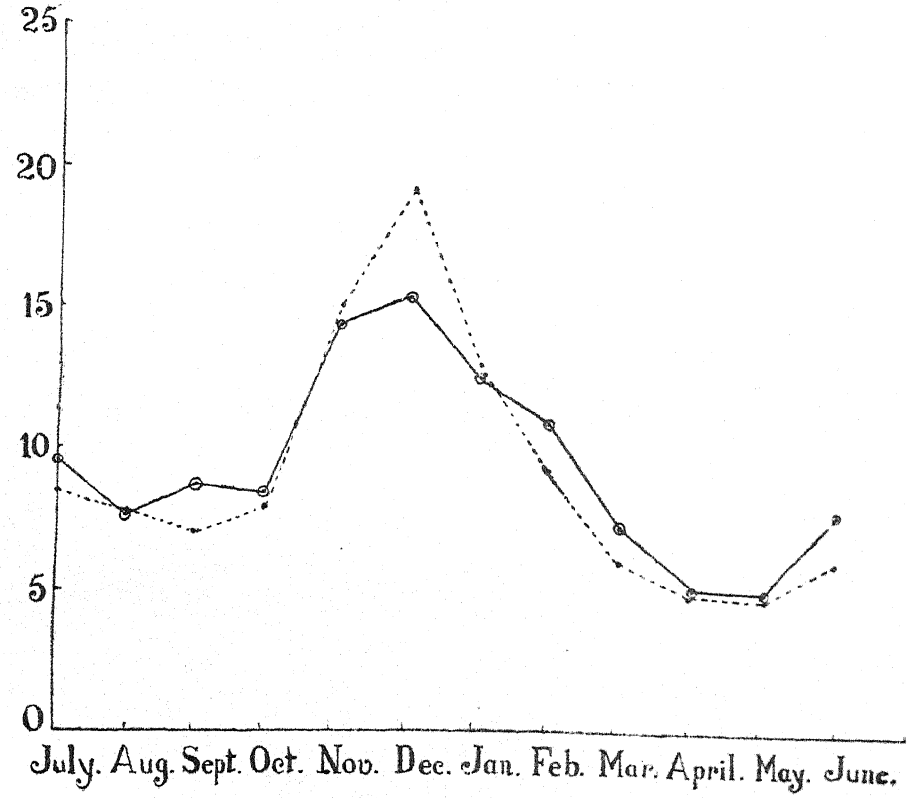


Fig. 4. Showing average longevity of adult.
Male:—Dotted line.
Female:—Continuous line.

Under laboratory conditions the maximum age of a female was 42 days in the month of December and the minimum one day in the months of March to October. The longest age of the male was 56 days in December and the shortest one day from February to September and in December.

The maximum average age for the female was 15·3 days in the month of December and minimum 4·8 days in May. The corresponding ages of the male were 19·0 days in December and 4·6 days in May. During the cold weather (November to January) the male is longer lived than the female; however, considered for the whole year the female appears to be slightly longer lived than the male, the yearly mean average age of the female and male being 9·3 and 9·0 days respectively. The subject is further discussed later.

TABLE III.

Longevity.

Month	Males				Females			
	Number confined	Minimum age in days	Maximum age in days	Average age in days	Number confined	Minimum age in days	Maximum age in days	Average age in days
July*	96	1	18	8·5	120	1	21	9·6
August	160	1	24	7·8	137	1	27	7·6
September	150	1	19	7·0	123	1	20	8·7
October	92	2	24	8·0	97	1	35	8·4
November	86	2	41	14·9	86	2	40	14·3
December	56	1	56	19·0	46	2	42	15·3
January	52	3	32	12·8	54	2	33	12·4
February	231	1	30	9·3	141	4	31	10·7
March	200	1	18	6·0	189	1	30	7·3
April	168	1	11	4·9	186	1	16	5·0
May	53	1	13	4·6	75	1	11	4·8
June	101	1	20	5·9	72	1	20	7·5

* *Vide* Misra, Negi, and Gupta [1930] July has been accepted as the first month of the year for the lac crops.

MATING.

The moths have never been noticed mating either in the field or in the laboratory. Presumably copulation takes place only in the night.

EGG-LAYING.

The predator laid eggs in the laboratory only in the cages provided with lac sticks and not otherwise. The eggs both in the field and the laboratory are generally laid either between the lac cells or in the anal openings of the female lac tests or in the empty tests of the male lac insects through the opercular hole. The eggs have also been found on the uncovered portions of the lac bearing branches, more

often on the portions which are covered with a black fungus commonly met with on the lac cultivated trees. In all cases the eggs are laid singly and never in groups.

The number of eggs laid was always higher in a cage where only two pairs of moths were confined than in the one in which more or less than two pairs were confined. This statement applies to cages of the sizes referred to under methods of study.

The preoviposition period and the duration of oviposition given in Table IV show that (1) the lowest preoviposition period was one day in August. November, December and March, highest 24 days in December and the yearly mean average 6·6 days ; (2) the duration of oviposition was lowest one day, and highest 20 days in November, and the yearly mean average 4·0 days.

FERTILITY.

Comparatively speaking this predator is less fertile than the predator *E. amabilis*. The largest number of eggs laid by an individual female was 142 in the month of November. The highest average *potential fertility* (Table IV) for a female was 70·0 in the month of December and lowest 37·0 in May, the yearly average being 54·0. The highest average *actual fertility* was 33·0 in the month of March and lowest 4·0 in December, the yearly average being 20·0.

The fertility figures in Table IV also show that (1) the average *potential fertility* is high in the months of November, December and March and is lowest in summer season (April-June); (2) the average *actual fertility* is high in the months of August, September, October, November and March and is lowest both in winter (December-February) and summer (April-June) seasons; (3) the *percentage of actual fertility* ranges high in the months of September, October, November, January, March and May, and (4) the percentage of egg-laying females is above the yearly average in the months of June to November.

Discussion on longevity, oviposition and fertility.

The predator has the hightest potential fertility at the advent of the winter season. *i.e.*, in the months of November and December and the next highest in the month of March which is followed by summer season. Both the seasons are unfavourable to oviposition and development of the predator. It is mainly by virtue of high potential fertility that the actual fertility ranges second highest in the month of November. Also that the moths may lay as many eggs as possible in this and the subsequent adverse cold months, the predator has longer life too. The predator, after passing through the unfavourable conditions of winter finds more favourable climatic conditions in March, and, therefore, to survive the next ordeal of the summer season, the moth in this month stands third in high potential fertility, and first in actual fertility and second in its percentage. However, the progeny of

these moths due to dry heat reaches the rainy season in much decreased numbers. The rainy season (July to September) affords the best conditions for the growth of the predator. Therefore, during this period although it has neither a high potential fertility nor a long life, it multiplies greatly by virtue of higher percentage of egg-laying females and comparatively speaking higher actual fertility and the percentage of actual fertility.

TABLE IV.

Preoviposition and oviposition period; fertility and percentage of egg-laying females.

Month		Preoviposition period in days	Oviposition period in days	*Actual fertility per individual	†Potential fertility per individual	Percentage of actual fertility per individual	Females under observation	Females laid eggs	Percentage of egg-laying females
July	Var.	2-13	1-11	1-73	4-109	26	39	28	72
	Aver.	6·7	4·0	14·0	54·0				
August	Var.	1-13	1-8	1-60	6-194	39	46	31	67
	Aver.	5·7	3·0	21·0	54·0				
September	Var.	2-13	1-18	1-81	3-165	49	48	38	79
	Aver.	6·0	3·7	27·0	55·0				
October	Var.	2-9	1-7	1-84	9-82	56	15	11	73
	Aver.	5·3	4·3	29·0	52·0				
November	Var.	1-16	1-20	1-142	10-131	48	24	14	58
	Aver.	6·7	6·0	32·0	66·0				
December	Var.	1-24	1-13	1-9	33-140	6	12	5	42
	Aver.	17·0	5·5	4·0	70·0				
January	Var.	2-10	1-10	1-46	3-151	43	30	5	17
	Aver.	5·0	5·0	18·0	42·0				
February	Var.	3-19	1-9	2-49	22-91	24	24	11	46
	Aver.	7·0	3·0	12·0	51·0				

* Eggs laid.

† Eggs laid + undelivered eggs.

Var. — Variation. Aver. — Average.

TABLE IV—*contd.*

Month		Preoviposition period in days	Oviposition period in days	*Actual fertility per individual	†Potential fertility per individual	Percentage of actual fertility per individual	Females under observation	Females laid eggs	Percentage of egg-laying females
March	Var.	1-14	1-16	1-110	12-225	52	45	21	47
	Aver.	5·7	4·3	33·0	63·0				
April	Var.	...	...	1-12	10-107	13	25	2	8
	Aver.	4·5	2·0	7·0	55·0				
May	Var.	4-4	1-8	7-30	7-98	51	14	2	14
	Aver.	4·0	5·0	19·0	37·0				
June	Var.	2-8	1-9	1-49	29-119	40	17	10	59
	Aver.	5·0	3·0	19·0	47·0				
Mean	Yearly	1-24	1-20	1-142	3-225	37	339	178	53
	Average	6·6	4·0	20·0	54·0				

* Eggs laid.
† Eggs laid + undelivered eggs.
Var. — Variation. Aver. — Average.

LIFE-HISTORY.

The life-cycle period of an insect is the time it takes to develop from an egg to the adult stage. But to find out generations of an insect the most suitable way would be to take the period from the day an egg is laid to the day the resulting female deposits eggs for the first time. The female after emerging from the pupa to the time it begins to deposit eggs is called by Uvarov [1931] "immature adult", but in order to avoid the division of the adult female into the immature and mature we have preferred to use the term 'preoviposition period' to this part of the adult famale's life.

The results of the life-history periods for the eggs laid during the various months of the year are tabulated in Table V. From the study of these results it will be seen that both the male and the female moths resulting from the eggs laid in June take the shortest time to complete their life-cycles; the period being 33·0 days for

the male and 32·0 days for the female. The life-cycle period for male as well as female is longest for the eggs laid in the month of September, 198 days for the male and 214 days for female. The high range of variation in the life-cycle periods for September eggs shows that for some hibernation starts in this month and others do not hibernate. The lower range of variation in the life-cycle periods and the longer average life-cycle periods for October, November and December eggs than of the September ones indicate that during these months hibernation is common. The male on the whole has a shorter life-cycle than the female.

TABLE V.

Life cycle.

Month		Duration of life stages of male in days					Duration of life stages of female in days				
		Egg	Larval	Pupal	Total life cycle	Average of	Egg	Larval	Pupal	Total life cycle	Average of
July	Var.	4-5	19-49	5-11	34-62	31	3-6	19-50	5-12	34-62	37
	Aver.	4·2	32·8	8·8	45·8		4·2	35·9	8·6	48·7	
Aug.	Var.	2-6	19-35	8-11	30-48	40	2-6	16-36	7-11	29-49	24
	Aver.	4·4	24·6	8·8	37·9		4·6	24·5	8·2	37·5	
Sept.	Var.	4-6	22-182	10-15	38-198	9	4-6	24-198	9-13	41-214	7
	Aver.	4·9	77·7	11·6	94·2		4·8	71·2	11·2	87·2	
Oct.	Var.	4-6	93-161	10-18	115-178	8	4-6	115-153	10-18	137-170	8
	Aver.	4·8	135·7	13·5	154·0		5·0	136·8	13·0	154·8	
Nov.	Var.	...	...	...	...	1	11-11	98-129	9-12	120-151	7
	Aver.	11·0	97·0	11·0	119·0		11·0	108·0	11·0	130·0	
Dec.	Var.	9-19	75-94	11-12	105-117	4	8-11	89-107	11-12	109-129	3
	Aver.	12·5	88·0	11·7	112·2		10·0	96·0	11·3	118·0	

TABLE V—*contd.*

Month		Duration of life stages of male in days					Duration of life stage of female days				
		Egg	Larval	Pupal	Total life cycle	Average of	Egg	Larval	Pupal	Total life cycle	Average of
Jan.	Var.	...	...	...	...	1	...	...	...	...	Mean of Dec. & Feb.
	Aver.	16·0	68·0	10·0	94·0		9·0	83·3	10·2	102·5	
Feb.	Var.	6-15	43-54	7-9	58-71	4	...	...	...	...	1
	Aver.	10·0	48·2	8·2	66·5		8·0	70·0	9·0	87·0	
Mar.	Var.	6-7	38-53	7-8	53-66	2	6-7	54-67	6-9	67-82	6
	Aver.	6·5	45·5	7·5	59·5		6·2	63·0	7·5	74·7	
April	Var.	5-7	31-47	7-8	43-61	6	5-6	43-47	7-8	56-60	2
	Aver.	5·5	41·3	7·3	54·1		5·5	45·0	7·5	58·0	
May	Var.	...	...	...	...	1	...	...	...	...	1
	Aver.	4·0	20·0	9·0	33·0		4·0	24·0	9·0	37·0	
June	Var.	3-4	23-45	6-12	33-60	13	3-4	23-34	6-11	32-47	14
	Aver.	3·7	30·1	9·4	43·2		3·6	26·6	8·8	39·0	

GENERATIONS.

The number of generations the predator has in a year have been calculated from the fifteenth of July [Misra, Negi and Gupta, 1930]. If the generations are calculated on the basis of the average life-cycle periods given in Table V *plus* the preoviposition period in Table IV for the month in which the life cycle is completed, the predator seems to have five generations in twelve months and twenty-five days (Fig. 5.). Even if it be assumed that in each month averages based on a larger number of life-cycles than in Table V may further lower the average life-cycle period by the

extent of the preoviposition period of each generation, and, therefore, if in calculating the generations the preoviposition period of each generation is left out of consideration, the predator would seem to have the same five generations in eleven months and twenty-eight days. If a series of generations is taken at an average rate of development for the calendar year sequence beginning with the first of January the predator will have the same five generations in a year's time. It will be clear, therefore, that the predator has on an average five generations in a year and not six as previously believed [Institute Annual Report, 1929-30 and Glover, 1931]. This is further supported by the results obtained from breeding of lineal generations.

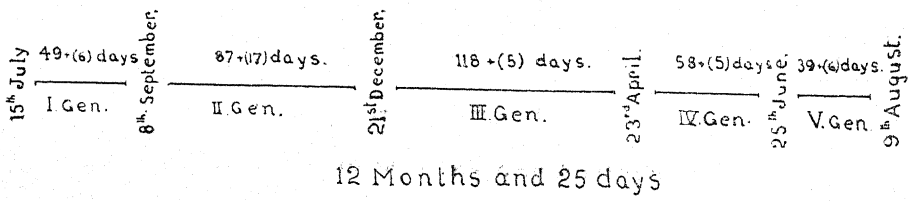


Fig. 5. Generations, (Life-cycle in ordinary numbers, preoviposition period in brackets.)

The life-cycle of the predator is not as simple as represented in Fig. 5. The complexity begins with the second generation. The eggs of this generation laid on the same dates in the month of September develop and a part emerges as adults in the month of October-November and the other part hibernates in the *Katki* stored and *Aghani** lacs, which continues to emerge during the succeeding months till April. Most of the eggs laid by the second generation on the *Baisakhi* and the *Aghani* crops do not develop due to cold but those which survive and develop further emerge as adults in the months of March and April. This is the third generation. The eggs laid by these moths emerge as adults in May and June. This is the fourth generation. The eggs laid by the females of this generation before the fifteenth of June generally do not develop due to heat, but those which survive emerge along with those laid in the second half of June in the months of July and August. This is the fifth generation. The third, fourth and part of the fifth generation develop under very trying conditions.

LINEAL GENERATIONS.

Attempts were made to find out the number of lineal generations the predator has in a year by direct breeding. Only two cases were partially successful. The

* Lac crop progeny of pure *kusum* brood, infected in June-July, matures in January-February.

one beginning on the 29th June 1931 ended on the 26th March 1932 and the other beginning on the 1st July 1931 ended on the 19th March 1932. In both of these the breeding could be carried out only up to the third generation, because in the first case the resulting third generation moth was a male and in the second, the third generation female failed to lay eggs. However, the three generations, which could be bred directly, confirm closely the statements made previously. If in these two cases the remaining generations for the year be completed by taking the average preoviposition and the life-cycle period for the corresponding months from Tables IV and V, it will be found that in both cases there would be only five generations in slightly over a year. This method would also indicate that the predator has only five generations in about a year's time.

Crop statistics.

Crop statistics for the prevalence of the predator in the various broods were collected as reported under methods of study, by weekly examination of sticks, and monthly caging of the stick lac in parasite boxes. For the *Aghani* and *Jethwi** crops in the absence of *S. trijuga* brood, the results of stick examinations and caging of immature lac are based on the progeny of *kusum* (*S. trijuga*) brood on *ber* (*Z. Jujuba*) and *khair* (*Acacia Catechu*) only.

Crop examinations.

The results of stick examinations are tabulated in Tables VI and VII. From a study of these results, it will be seen that the attack of the predator like that of the predator *E. amabilis* is more on the *Aghani* and *Katki* (July-February) than on the *Jethwi* and *Baisakhi* (November-July) crops. In each season the former of each group is affected more than the latter, the average number of the predator per 100 in. being 73·5 in *Aghani*, 72·0 in *Katki*, 36·5 in *Jethwi* and 21·6 in *Baisakhi*.

Further if *Aghani* and *Katki* are compared during September, October and November, the latter is found more affected than the former, and in March and May *Baisakhi* is affected more than *Jethwi*, but if averages are taken for the months (July to November for *Aghani* and *Katki* on one side and February to July for *Jethwi* and *Baisakhi* on the other) during which the crops of each set grow side by side in the fields the ratios of predator's prevalence would be *Katki* 18 : *Aghani* 13 : *Jethwi* 9 : *Baisakhi* 8.

* Lac crop progeny of the *kusum* brood, infected in January-February, matures in June-July.

TABLE VI.

Crop examinations.

Katki *1927 to 1931 and* Aghani *1927-28 to 1931-32. Number reduced to per 100 ft. length of stick.*

Months		Eggs		Larvae	Pupae		Total
		Unhatched	Hatched		Full	Empty	
July	*Katki*	20·4	..	..	..	..	20·4
	Aghani	..	..	..	..	..	..
August	*Katki*	385·2	136·8	122·4	..	..	644·4
	Aghani	482·4	112·8	198·0	..	..	793·2
September	*Katki*	206·4	351·6	495·6	19·2	15·6	1,088·4
	Aghani	171·6	122·4	374·4	..	..	668·4
October	*Katki*	216·0	439·2	578·4	62·4	34·8	1,330·8
	Aghani	33·6	162·0	372·0	22·8	45·6	636·0
November	*Katki*	39·6	240·0	879·6	..	79·2	1,238·4
	Aghani	..	62·4	846·0	124·8	31·2	1,064·4
December	*Aghani*	..	468·0	976·8	25·2	42·0	1,512·0
January	*Aghani*	..	190·8	903·6	69·6	34·8	1,198·0
February	*Aghani*	..	144·0	620·4	164·4	20·4	949·2
March	*Aghani*	..	..	342·0	342·0	428·4	1,112·4
Total	*Katki*	867·6	1,167·6	2,076·0	81·6	129·6	4,322·4
	Aghani	687·6	1,262·4	4,633·2	748·8	602·4	7,934·4

TABLE VII.

Crop examinations.

Baisakhi *1927-28 to 1931-32 and* Jethwi *1928 to 1931. Number reduced to per 100 ft. length of stick.*

Months		Eggs		Larvae	Pupae		Total
		Unhatched	Hatched		Full	Empty	
November	*Baisakhi*	..	..	..	..	..	..
December	*Baisakhi*	2·4	2·4	..	..	..	4·8
January	*Baisakhi*	2·4	..	..	..	..	2·4
February	*Baisakhi*	21·6	9·6	..	..	..	31·2
March	*Baisakhi*	51·6	9·6	60·0	..	..	121·2
	Jethwi	..	10·8	10·8	..	..	21·6
April	*Baisakhi*	20·4	147·6	277·2	..	1·2	446·4
	Jethwi	86·4	337·2	250·8	..	..	674·4
May	*Baisakhi*	9·6	159·6	403·2	12·0	9·6	594·0
	Jethwi	..	169·2	326·4	..	..	495·6
June	*Baisakhi*	20·4	86·4	235·2	36·0	18·0	396·0
	Jethwi	50·4	141·6	168·0	76·8	..	436·8
July	*Baisakhi*	151·2	184·8	312·0	19·2	70·8	738·0
	Jethwi	39·6	339·6	499·2	99·6	19·2	997·2
Total	*Baisakhi*	279·6	600·0	1,287·6	67·2	99·6	2,334·0
	Jethwi	176·4	998·4	1,255·2	176·4	19·2	2,625·6

STORAGE OF LAC.

The immature lac was caged in the form of lac sticks and the mature as scraped lac, therefore, to make the results of each month comparative in Tables VIII and IX. The weight of mature lac has been reduced to lengths of lac sticks on the basis of 4·5 grms. of scraped lac to one foot of lac stick. This basis has been arrived at from the scraped lac obtained from about six miles of *Katki*, *Baisakhi* and *Jethwi* lac sticks.

The results of emergence from parasite boxes, recorded in Tables VIII and IX also corroborate that the *Aghani* and *Katki* as a whole are affected more than *Jethwi* and *Baisakhi*. They also show that *Aghani* is affected more than *Katki* and *Jethwi* more than *Baisakhi*. A heavy emergence of the predator in the months of September, October-November, February-April, May-June and July-August roughly suggests five generations in a year. A sudden rise in the emergence in March from the lac caged in October suggests hibernation in winter season. The continuous emergence for four or five months from the lac caged in months other than winter or a few pre-winter months confirms the fact that the moth is capable of breeding on dead lac insects and the dry lac. The emergence in large numbers after four to five months and not immediately after the maturity of the *Katki* crop in October-November suggests that the control of the predator would be easier in localities where only the *Katki* and *Baisakhi* crops are grown.

TABLE VIII.

Storage of lac.

Katki *1926 to 1931 and* Aghani *1926-27 to 1931-32. Emergence reduced to per 100 ft. of stick lac.*

Caging months / Emergence months		August	September	October	November	December	January	February	March	TOTAL
August	*Katki*	0·00								
	Aghani	0·00								
September	*Katki*	15·00	14·00							
	Aghani	46·30	23·94							
October	*Katki*	7·50	60·70	5·30						
	Aghani	0·00	108·45	36·74						

TABLE VIII—*contd.*

Caging months / Emergence months		August	September	October	November	December	January	February	March	TOTAL
November.	*Katki*	0·00	47·00	28·30	0·03					
	Aghani		7·04	17·84	0·00					
December.	*Katki*		0·00	11·00	1·05	0·02				
	Aghani		0·00	0·00	1·07	1·42				
January .	*Katki*			1·30	0·39	0·16	0·00			
	Aghani		1·40	4·20	0·53	2·84	2·16			
February .	*Katki*			2·80	9·10	11·26	9·00			
	Aghani		0·00	12·59	11·33	19·92	42·05	3·81		
March .	*Katki*			51·00	54·93	65·91	0·00			
	Aghani		2·81	161·68	69·60	145·19	143·93	42·34	21·06	
April .	*Katki*			18·30	17·30	19·87	13·66			
	Aghani		1·40	10·50	11·33	39·85	44·22	11·70	13·70	
May .	*Katki*			0·10	0·84	1·34	9·09			
	Aghani		0·00	0·00	0·00	1·42	0·43	0·26	0·52	
June .	*Katki*			0·00	0·01	0·00	0·00			
	Aghani					0·00	0·06	0·00	0·00	
July .	*Katki*			0·00	0·46	0·00	4·54			
	Aghani						0·00	0·01	0·00	
August .	*Katki* .				0·08	0·04	2·27			
	Aghani							0·00	0·00	
September	*Katki* .				0·00	0·00	0·75			
	Aghani							0·00		
October .	*Aghani*						0·00			
Total .	*Katki*	22·50	121·70	118·10	84·19	98·60	30·31			475·40
	Aghani	46·30	145·04	243·55	93·86	210·64	232·79	58·12	35·28	1065·58

TABLE IX.

Storage of lac.

Baisakhi *1926-27 to 1930-31 and* Jethwi *1927 to 1931. Emergence reduced to per 100 ft. of stick lac.*

Caging months / Emergence months		December	January	February	March	April	May	June	July	August
December.	*Baisakhi* .	0·00								
January .	*Baisakhi* .	0·00	0·00							
February .	*Baisakhi* .	0·00	0·00	0·00						
March .	*Baisakhi* .		0·00	0·00	0·00					
	Jethwi .				0·00					
April .	*Baisakhi* .		0·00	0·00	1·75	9·39				
	Jethwi .				0·00	0·00				
May .	*Baisakhi* .			0·00	1·75	12·08	2·18			
	Jethwi .					0·95	0·00			
June .	*Baisakhi* .			0·00	1·40	2·68	12·85	9·76		
	Jethwi .					0·95	26·66	17·74		
July .	*Baisakhi* .			0·00	0·00	0·00	5·91	15·08	4·20	
	Jethwi .					0·00	8 88	0·00	4·46	
August .	*Baisakhi* .					0·00	3·34	12·38	40·44	52·38
	Jethwi .					3·82	0·00	14·81	103·94	2. ·86

TABLE IX—*contd.*

Caging months / Emergence months		December	January	February	March	April	May	June	July	August
September	*Baisakhi*						0·77	6·28	29·63	24·24
	Jethwi					0·00	..	0·00	26·65	25·39
October	*Baisakhi*						0·51	1·91	7·07	13·46
	Jethwi								4·69	4·88
November	*Baisakhi*						0·00	0·26	0·89	7·51
	Jethwi								1·04	0·53
December	*Baisakhi*							0·00	0·18	0·97
	Jethwi								0·22	0·12
January	*Baisakhi*								0·01	0·08
	Jethwi								0·07	0·00
February	*Baisakhi*								0·00	0·08
	Jethwi								0·07	0·00
March	*Baisakhi*									2·47
	Jethwi								0·00	0·00
April	*Baisakhi*									0·44
	Jethwi								0·00	
May	*Baisakhi*									0·00
Total	*Baisakhi*	0·00	0·00	0·00	4·90	24·15	25·56	55·67	82·33	101·63
	Jethwi				0·00	5·72	35·54	32·55	141·14	60·78

HIBERNATION.

The predator hibernates in the larval stage from September but mostly from October-November to March. The larvæ at this time are not very active. During the winter months the predator has even its other stages of life besides the larval more prolonged than in other months.

DISTRIBUTION.

The moth is almost as widely distributed all over India as *Eublemma amabilis*. It was reared from lac received from Halimahat, Sonapur, Palas-Bari and Garo Hills (Assam); Ranchi, Manbhum, Singbhum, Dumka, Hazaribagh, Daltongunj, Pusa and Sambalpur (Bihar and Orissa); Mathurapur, Pakur, Nimtita, Sibgunj and Raghunathgunj (Bengal); Benares, Kheri, Dehra Dun and Meerut (U. P.); Damoh, Raipur, Kota, Bilaspur, Jubbulpore and Rewa (C. P.); Panna State (C. I.); Hyderabad Deccan; Kashmir; Jodhpur; Bangalore (Mysore); Salem District (Madras); Dharwar and Bijapur (Bombay); Lyallpur (Punjab) and Mimbu, Maymya, Taunggyi, Hwe, Hish seng, Maymyo Reserve, Nampan District and Shan States (Burma).

ECONOMIC STATUS.

The predator damages both the lac crops in the field and the stored lac in the godowns like the predator *E. amabilis*. During the larval life it is capable of devouring from 7 to 45 mature female lac cells. In addition it does mischief by giving stray bites to the neighbouring lac cells when trying to enter in the lac incrustation or while moving about after entering it. It is, therefore, highly destructive both to the lac insect and the stick lac. It will be seen from Table X that it is not so injurious to the fresh lac in the field as the predator *E. amabilis* though on the average it has 47·0 days larval life (average of 256) while *E. amabilis* has only 35·3 days (average of 177), but in the destruction of stored lac it surpasses the predator *E. amabilis*. It should, therefore, be taken as seriously as *E. amabilis*. The figures given in Table X are from the same length examined and from the same quantity of lac stored for about three years in both cases.

TABLE X.

Comparative presence of H. pulverea *and* E. amabilis.

In the same length of fresh lac stick		From the same amount of stored stick lac	
H. pulverea	*E. amabilis*	*H. pulverea*	*E. amabilis*
964	4,044	3,576	3,382

Natural enemies.

Known.

These are arranged more or less in order of their prevalence :—

1. Formicoidae.—The ants *Camponotus compressus* Fabr. and *Solenopsis geminata* sub sp. *rufa* Jerdon [Negi, Misra, Gupta, 1930] pick up *Holcocera pulverea* larvæ when they come out of the eggs and try to enter into the lac cell and also pick up the exposed larvae and pupae of the predator.

2. Braconidæ.—*Apanteles tachardiæ* Cam. is an internal parasite of the nearly mature larva of the predator. The parasitised larva in the early stages shows no signs of parasitisation, but a day before the exit of the parasite larva from its body it becomes lethargic and slightly lighter in colour. The parasite larva comes out of the body of the host by biting a hole either in the side of the host or in the dorsal soft portion between the head and pro-thoracic shield. It begins to spin the cocoon in the gallery of the host after it has partly left its body. When the cocoon is half spun, it leaves the host completely and enters the cocoon keeping its head towards the open end. After completing the cocoon it pupates. The parasite larva takes about two hours to prepare its cocoon. The cocoon measures 3·5-4·5 mm. in length and 1-1·5 mm. in width. It is most abundant in February-March. Only one parasite larva is met with in each host. The adult comes out by raising the lightly fixed cap of the cocoon.

3. Ichneumonidæ.—*Pristomerus testaceicollis* Cam. [Institute Annual Report, 1931-32] is an internal parasite of the larva. It also parasitises the nearly fully grown larva which does not show any sign of parasitisation till a day or two before the parasite larva is due to leave the host. It comes out of the body of the host by cutting a hole on the ventral side of the pro-thorax and after it has left the host completely spins its cocoon close to the remains of the host. Only one larva develops in the body of each host. The cocoon is papery in texture and brownish white in colour, it measures 6-8 mm. in length and 1·5-2·5 mm. in width. It is mostly met with in November, December, February and March. The adult emerges by cutting a hole in the cocoon.

4. Bethylidæ.—*Bethylid sp.* is an external larval parasite. It lays eggs on the larva after paralysing it. As many as eight eggs have been met with on a single larva. The larvæ when fully fed spin loose dirty white cocoons attached to one another. Each cocoon is about 0·7-1·0 mm. broad. The adult seems to be of nocturnal habits as it tries to hide itself in dark and shady places in the day time. It is very active and the male smaller than the female. It is most abundant in February-March and is mostly met with in the *Aghani* crops.

5. *Chalcidiidæ.—Brachymeria tachardiæ* Cam. is an endoparasite of the pupæ of both *H. pulverea* and *E. amabilis*, and not to our present knowledge an ecto-parasite of the larva as stated by an oversight by Glover [1930]. The adult emerges by biting a circular hole about the middle of the body of the host. The parasites bred from *H. pulverea* pupæ are smaller in size than those from *E. amabilis*, otherwise both resemble in all other respects.

6. *Eurytoma palladiscapus* Cam. is also an endo-parasite of the pupa.

7. *Brasema annulicaudis* Cam. only one case of an endo-parasite of the pupa has so far been met with. This chalcid is both a friend and an enemy to the lac insect. But its important role is that of an enemy rather than of a friend because it is primarily an ecto-parasite of the larva of *Bracon tachardiæ* Cam., the chief enemy of the predator *E. amabilis* and an endo-parasite of the lac insect itself.

8. *Pyralidæ.—Ephestia sp.* mainly a scavenger but its larva always attacks *Holcocera* larva or pupa when it comes in contact with it in its galleries.

9. *Braconidæ.—Bracon tachardiæ* Cam. is an ectoparasite of the larva though extremely rare (Insectary cage slip No. 558/31 'I' and 586/31 'I').

Suspected.

1. *Braconidæ.—Apanteles fakhrulhajiæ* Mahdn.

All the above enumerated enemies of the predator, except Nos. 1 and 8 attack it when it has done a considerable amount of damage. In view of this and the fact that the predator breeds freely in the stored lac, Negi, Gupta and Misra [1931] are of opinion that the biological control of this predator and *E. amabilis* is less likely to succeed substantially.

CONTROL MEASURES.

The most important of the artificial measures are fumigation and water immersion but they are not described in this paper because the effect of these treatments on the properties of shellac obtained from the treated material is still under investigation and it is hoped that they will be discussed separately in the near future. The other control measures are as follows :—

1. At the maturity of the crop, all the crop but the portion to be used as brood should be scraped soon after reaping from the field, and the same should be done with the brood lac after the infection is over. The prompt scraping will by

itself kill a good number of the enemies of the lac insect, and the exposed stages of the enemies in the scraped lac will be removed by ants [Negi, 1932].

2. Selected and, as far as possible, predator and parasite free brood should be used for infecting every crop. Self infection should be avoided [Misra, Negi and Gupta, 1930].

3. The brood lac should be removed from the trees as soon as they have received proper infection, but in no case should it be allowed to remain on the trees for more than three weeks, as practically all the lac larvæ swarm out by this time [Misra, 1929].

4. *Kusum* brood should be avoided to inoculate any other host but *kusum* and *khair* for the winter crop (July infection), because its progeny matures in January-February about three to four months later than the *Katki*. This additional exposure to the enemies of the lac insect and its resin naturally causes further damage to the *Aghani* crop. The newly infected *Baisakhi* crop which is practically free from the attack of the predators and parasites till January also gets infection from the *Aghani* crop, because the enemies of the lac insect emerge in larger numbers from the *Aghani* crop in February-March and they go and attack the *Baisakhi* crop standing in the field at a time which is favourable for the propagation of these insects.

Summary.

Holcocera pulverea is a moth predaceous on the lac insect. It also breeds on the dead lac insect and "*Phunki*" lac. It has five generations in a year, and does more injury to the *Katki* and *Aghani* than *Baisakhi* and *Jethwi*. The predator hibernates during the winter in larval stage. The female moth has a longer life cycle and age than the male. The longevity, oviposition period potential and actual fertility are interrelated and vary according to the favourable or unfavourable seasons of the year. The larva of the predator has five instars in each generation from July to October and nine from November to June. The predator is less prevalent in the field than the predator *E. amabilis* but more in the stored lac. It has several natural enemies but the biological control of the predator at present seems to be difficult.

The predator can be controlled to some extent if all the crop except the portion to be used as brood is scraped soon after reaping and the same is done with the brood lac after the infection is over.

Selected and, as far as possible, parasite and predator free brood should be used for every crop.

The brood should not be kept on the plants for more than three weeks in any case.

Kusum brood should be avoided to infect other host plants than *kusum* and *khair* in June-July.

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Key to lettering in figures 1—3.

a. . . . . Clypeus.
ab. . . . . Abdomen.
abc and ao . . . . Common aperture of bursacopulatrix and oviduct.
af i and ii . . . . Adfrontal seta No. 1 and No. 2.
af p. . . . . Adfrontal pore.
an. . . . . Anus.
ant. . . . . Antenna.
c. . . . . Cocoon.
cl. . . . . Clasper.
cr. . . . . Crochets.
cu. . . . . Cucullus.
d en. . . . . Damaged incrustation.
e. . . . . Egg.
ep. . . . . Epicranium.
f. . . . . Frons.
fcs. . . . . Fronto clypeal suture.
fl. . . . . Femur of the prothoracic leg.
fp. . . . . Frontal pore.
fr. . . . . Front.
ga. . . . . Male genital aperture.
gle. . . . . Glazed eye-piece.
h. . . . . Head.
h en. . . . . Healthy incrustation.
hr. . . . . Harpes.
j. . . . . Juxta.
lb p. . . . . Labial palp.
lbr. . . . . Labrum.
lg 1-lg 3 . . . . Legs.
md. . . . . Mandible.
mi. . . . . Micropyle.
mxp. . . . . Maxillary palps.
mx. . . . . Maxilla.
oc. . . . . Ocelli.
op c. . . . . Opening of cocoon.
p. . . . . Peniculus.
pl. . . . . Proleg.
s. . . . . Lac stick.
se. . . . . Sculptured eye.
sp. . . . . Spiracle.
th. . . . . Thorax.
tl. . . . . Thoracic leg.
un. . . . . Uncus.
v. . . . . Veniculum.
w. . . . . Wing.

THE MOVEMENT OF TOBACCO MOSAIC VIRUS IN LEAVES OF *NICOTIANA SYLVESTRIS**.

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(With Plates LVI and LVII.)

The literature dealing with the movement of viruses within the plant has been summarised by Henderson Smith [1930] and recently by K. M. Smith [1933]. It has been suggested that the spread of a virus within a plant may be effected through the vascular bundles or by diffusion from cell to cell. Experiments by Bennett [1927] and by Caldwell [1930] point to the translocation of the viruses through the phloem, whilst Grainger [1933] has come to the opposite conclusion that ordinary tobacco mosaic virus is capable of moving across a barrier of a steamed part of a tobacco stem. A number of investigators have also measured the rates of movement of the viruses within their hosts. Although dissemination by diffusion has been recognised as a means by which the virus may spread within its host, no direct experiments have been made in this direction, nor has the rate of spread been determined by this mechanism. The experiments described in this paper deal chiefly with the rate at which ordinary tobacco mosaic virus can move in its host independently of the translocation and transpiration streams.

MATERIALS AND METHODS.

The virus used in these experiments was of ordinary tobacco mosaic. The infective extract was prepared by grinding diseased leaves, cut into small pieces by sterile scissors, in a mortar ; to this 3 c.c. of water was added for each one gram of tissue and grinding continued until leaves were reduced to a very fine pulp, which was then squeezed through cheese-cloth. According to Henderson Smith [1928], the juice thus treated is diluted 1 in 8 to 1 in 10 in the case of aucuba or yellow mosaic of tomato. One may reckon the tobacco mosaic juice, receiving a similar treatment, to give nearly the same dilution.

* This work was done in the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research, Princeton, New Jersey, U. S. A.

The plants found most useful for these experiments were *Nicotiana sylvestris* Spegaz. & Comes, since the epidermis of these plants can be stripped off with little difficulty. It is easier to remove the lower than the upper epidermis, since the spongy parenchyma is a loose tissue with fewer points of attachment to the lower epidermis than the compact palisade tissue below the upper epidermis. Ordinary tobacco mosaic is systemic in *Nicotiana sylvestris* and causes symptoms similar to those in tobacco [Holmes, 1932].

Inoculation was made by rubbing the upper surface of the leaves with a cloth-covered glass spatula soaked in the virus extract, and in so doing special care was exercised that the inoculum did not run down the margins to the lower surface. By applying gentle friction to produce mechanical injury, it was possible to break the epidermal hairs without visibly injuring the epidermis. After appropriate intervals the lower epidermis was stripped from the loose mesophyll, and was lifted bit by bit by means of sterile, sharp-pointed forceps. Each portion of the epidermis was cut with sterile scissors from the end held by the forceps and was deposited in a few drops of water on a glass slide or in a Petri dish. Between each operation the forceps and scissors were sterilised in boiling water for one minute or longer. When a sufficient number of epidermal strips had been collected in this manner, they were macerated with a sterile glass spatula, and the resulting juice was inoculated to healthy bean plants, *Phaseolus vulgaris* L. var. Early Golden Cluster, about 10 to 12 days old. The first two leaves of each plant were inoculated. In a few cases *Nicotiana glutinosa* L. was used as a test plant. In the latter case the growing tip, the small top leaves and the old leaves at the base were removed, leaving five leaves for inoculation. Ordinary tobacco mosaic produces local necrotic lesions in both the bean and *Nicotiana glutinosa*.

All plants were grown in 4-inch clay pots, and after inoculation were removed to a greenhouse bench of peat moss which was kept well soaked with water. Inoculum was applied with a glass spatula, while the leaf was held on a piece of wax paper by the left hand. The temperature of the greenhouse ranged from 75 to 80° F., and on very warm days often rose to 82° F.; it seldom fell below 70° F.

In the case of test plants inoculation was made to a half-leaf, the other half being left untreated as a control. Final results were usually recorded 6 to 7 days after inoculation. None of the control half-leaves ever showed any infection.

Experimental work.

Early in this work it was necessary to work out a method to detect the presence of ordinary tobacco-mosaic virus in the lower epidermal layer after the leaf had been inoculated on the upper surface. Two methods were tested. In one case

strips of lower epidermis were macerated on a sterile microscopic slide with a glass spatula, and the resulting juice was inoculated to one side of bean leaves. In the other case the lower surface of the inoculated leaf was rubbed over gently for a number of times with a cloth-covered glass spatula soaked in water, after which the same spatula was used to inoculate the other half of the bean leaves. It was intended by the latter method to pick up the virus from the hairs and epidermal cells without causing injury to the mesophyll below.

It will be seen from Table I that the virus passed into the lower epidermal cells in sufficient quantities to be detected 48 hours after inoculation of the upper surface, and that its concentration increased as the time progressed. Although the virus was present in the lower epidermis after two days, it was not possible to pick it up in measurable quantities by rubbing the surface with a cloth-covered glass spatula

In order to study further the rate of spread of the virus, three leaves on each of six plants of *Nicotiana sylvestris* were inoculated on the upper surface. After appropriate intervals, ranging from 24 hours to 120 hours after inoculation, a leaf was cut out from a plant, and the lower epidermis was removed bit by bit and prepared for inoculum in the usual manner. *Nicotiana glutinosa* was used as a test plant, and the inoculum was applied to a half-leaf, the other half being left untreated as a control.

TABLE I.

Spread of ordinary tobacco mosaic virus in leaves of Nicotiana sylvestris.

Intervals	No. half-leaves of beans inoculated in each case	Total No. lesions	
		Epidermis	Rubbing
Hours			
18	9	0	0
24	11	0	0
48	12	41	0
72	12	110	0
96	12	230	0
120	12	264	0

The results of this experiment show that the virus can be detected in measurable quantities in the lower epidermis 40 hours after inoculation of the upper surface of the leaf, although there was only one lesion produced for every three inoculated leaves. There was not much increase in the concentration of the virus until 72 hours, after which the virus increased fairly rapidly in concentration. (Table II and Plate LVI, fig. A.)

TABLE II.

Rate of spread of virus in leaves of Nicotiana sylvestris.

Intervals	No. half-leaves of *Nicotiana glutinosa* inoculated	Total No. lesions
Hours		
24	10	0
28	15	0
32	15	0
36	15	0
40	15	5
44	15	17
48	10	3
72	15	137
96	15	465
120	15	1,087
144	15	1,185

The above experiment was repeated using the bean as a test plant. The results support the general conclusions of the last experiment, although in this case the virus was detected in the lower epidermal cells 36 hours after inoculation. They further show that there is little increase in the concentration of the virus between 36 and 56 hours, but after the latter period, the virus increases steadily in concentration. (Table III.) There were, however, fewer lesions produced in bean leaves, as compared with *Nicotiana glutinosa*, after the 72-hour test. (Tables III and IV; Plate LVI, fig. B.)

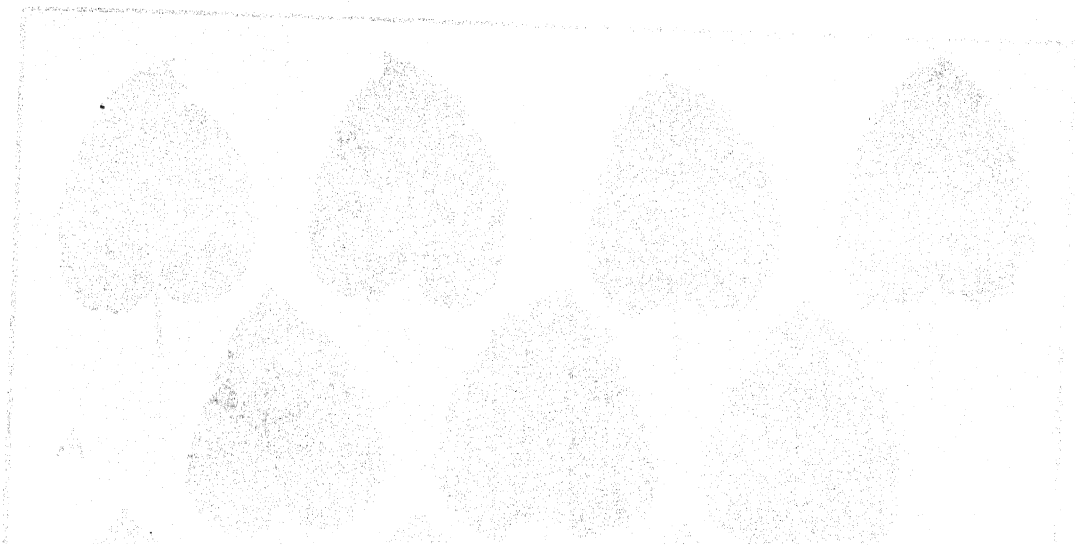
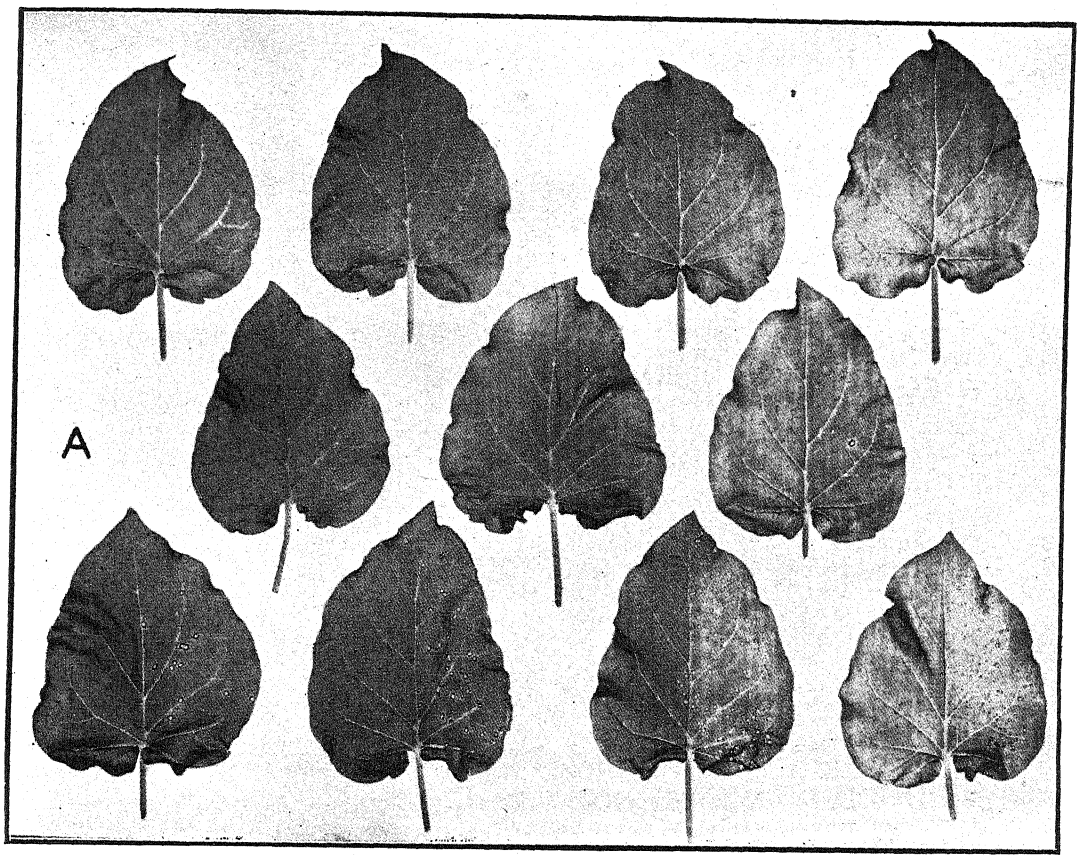


PLATE LVI.

Fig. *A*. Leaves of *Nicotiana glutinosa* showing lesions produced by inoculation with juice obtained by macerating strips of lower epidermis of leaves of *Nicotiana sylvestris* at varying intervals. From left to right. *Top line:* 24, 28, 32, 36 hours; *middle line:* 40, 44, 48 hours. *bottom line:* 72, 96, 120, 144 hours.

Fig. *B*. Same as in *A* above on bean leaves. From left to right. *Top line:* 48, 72, 100 hours; *bottom line:* 124, 144, 168 hours.


A

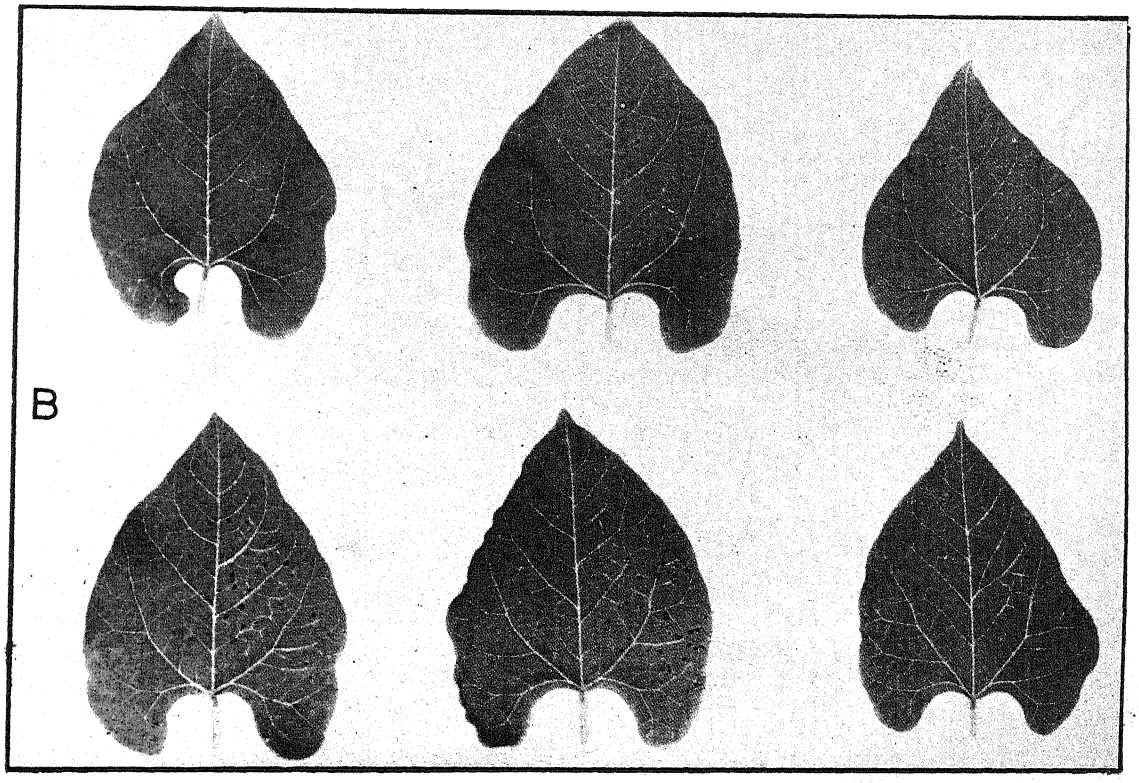

B

TABLE III.

Rate of spread of virus in leaves of Nicotiana sylvestris.

Interval	No. half-leaves of beans inoculated	Total No. lesions
Hours.		
24	12	0
28	12	0
32	12	0
36	12	8
40	12	8
44	12	6
48	12	8
52	12	10
56	12	10
60	12	46
64	12	68
68	12	107
72	12	97
96	12	203
120	12	322

TABLE IV.

Rate of spread of virus in leaves of Nicotiana sylvestris.

Interval	No. half-leaves of beans inoculated	Total No. lesions
Hours.		
48	12	2
72	12	39
100	12	80
124	12	303
144	12	716
168	12	349

Another experiment was made, in which strips of lower epidermis were tested in the usual manner at intervals of two hours between the period of 28 and 38 hours after inoculation. It will be seen from Table V that the virus was not detected in the epidermis until 38 hours after inoculation, but it seems unnecessary to lay much emphasis on this point, since the method of detecting the virus in the epidermal cells is only relative as the rate at which the virus travels in the leaf tissue

is likely to change somewhat due to variations in the thickness of the lamina of the leaf and the temperature to which the plants are exposed during the test.

TABLE V.

Rate of spread of virus in leaves of Nicotiana sylvestris.

Interval	No. half-leaves of beans inoculated	Total No. lesions
Hours.		
28	12	0
30	12	0
32	12	0
34	12	0
36	12	0
38	12	2

The foregoing experiments indicate that the virus travels from the upper to the lower epidermis of the leaf of *Nicotiana sylvestris* in 36 to 40 hours, but it was not clear for what portion of this time the virus is actually moving in the leaf tissue, or, in other words, how long it takes the virus to pass from the epidermis into the mesophyll below when the leaf has been inoculated on the lower surface. Inoculation was made on the lower surface, since it is difficult to strip the upper epidermis from the underlying tissue. Accordingly three leaves of each of two plants of *Nicotiana sylvestris* were inoculated on the lower surface. After appropriate intervals ranging from 4 to 20 hours after inoculation, the lower epidermis was carefully removed with sterile forceps, and blocks of tissue comprising the mesophyll and upper epidermis were cut out with a sterile knife from the centre of a region where the lower epidermis had been carefully removed; special care was taken not to include any portion of lower epidermis. The blocks from each leaf were macerated in a few drops of water in a Petri dish, and the resulting juice was inoculated to test plants of *Nicotiana glutinosa*.

It will be seen from Table VI that the virus passed into the mesophyll below the lower epidermis in 12 hours after inoculation. The test after 14 hours, however, gave negative results whilst there was enough evidence that the virus had passed in sufficient concentration into the mesophyll after 16 hours. (Plate LVII, fig. A.)

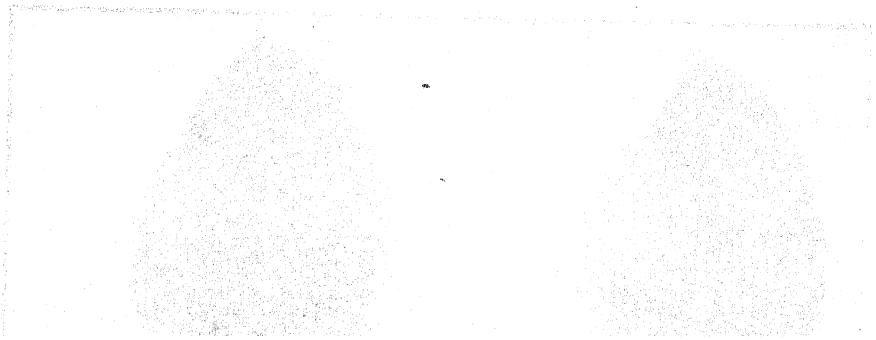
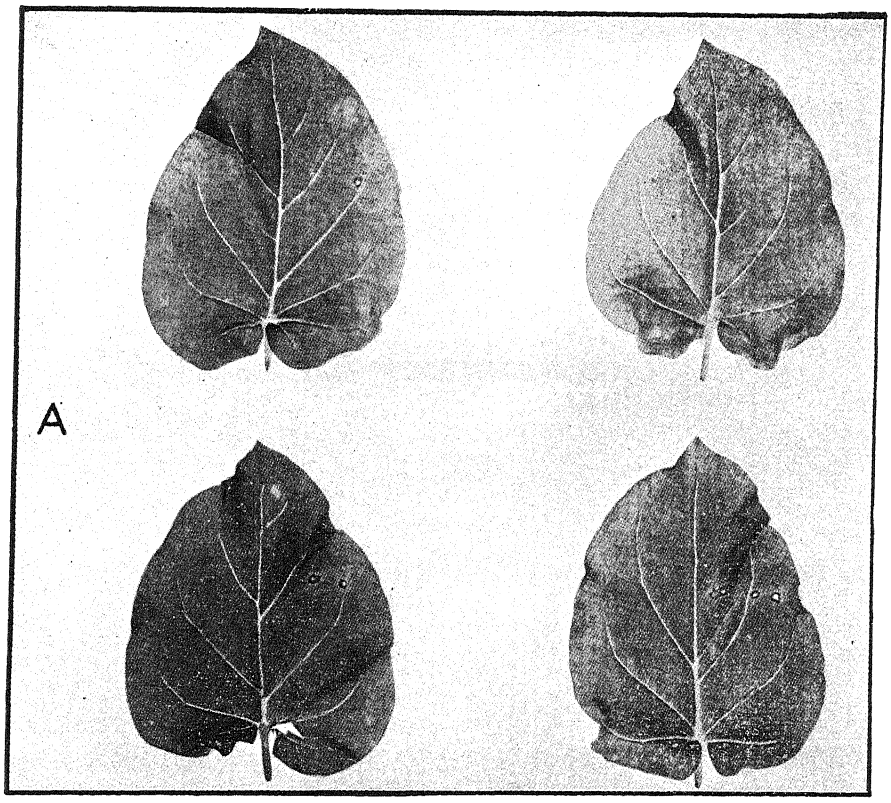
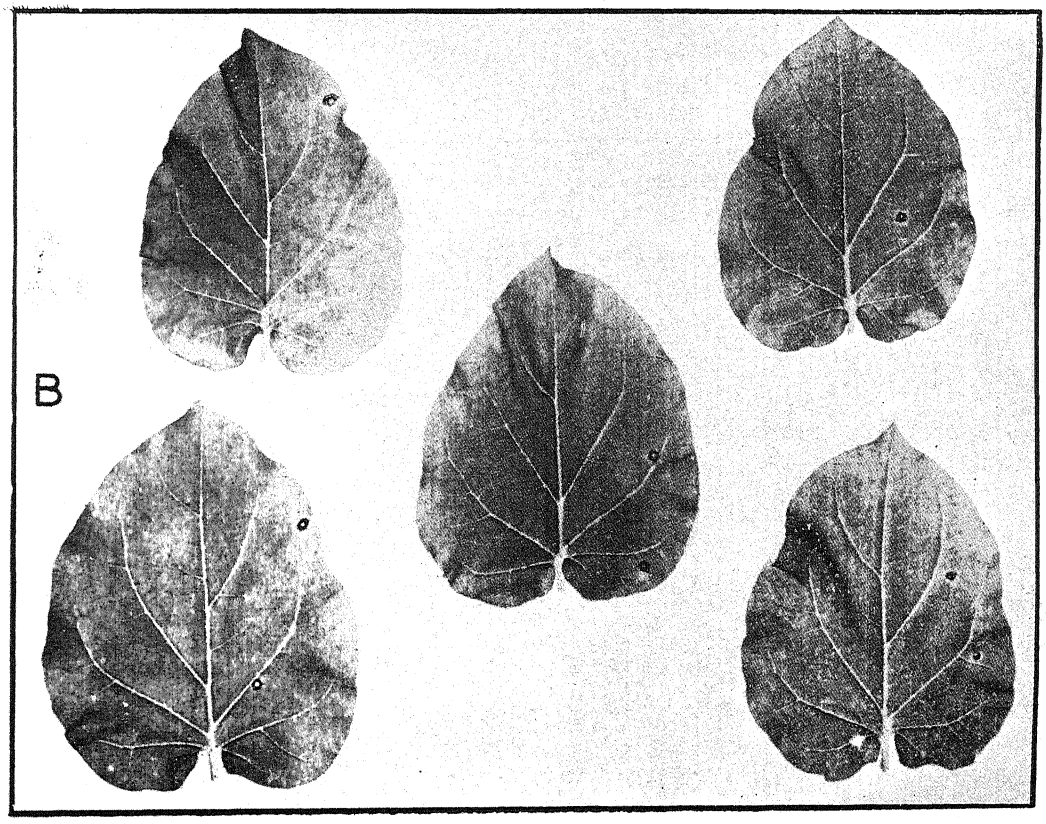


PLATE LVII.

Fig. *A*. Leaves of *Nicotiana glutinosa* showing lesions produced by inoculation with juice obtained by macerating blocks of mesophyll and upper epidermis of leaves of *Nicotiana sylvestris* at varying intervals. From left to right. *Top line:* 12, 14 hours; *bottom line:* 16, 18 hours.

Fig. *B*. Same as in *A* above of this plate. From left to right. *Top line:* 4, 6 hours; *middle line:* 9 hours; *bottom line:* 10, 12 hours.


A


B

Another test was made of the period elapsing between inoculation and the appearance of the virus in the mesophyll below the epidermis. The intervals tested ranged from 4 to 15 hours after inoculation. The results in Table VII show that the virus can be detected in very low concentration in the mesophyll as early as 4 hours after inoculation. (Plate LVII, fig. B.)

TABLE VI.

Interval elapsing between inoculation and appearance of virus in mesophyll below epidermis.

Interval	No. half-leaves of *Nicotiana glutinosa* inoculated	Total No. lesions
Hours.		
12	10	2
14	10	0
16	10	10
18	10	21
20	10	10

TABLE VII.

Interval elapsing between inoculation and appearance of virus in mesophyll below epidermis.

Interval	No. half-leaves of *Nicotiana glutinosa* inoculated	Total No. lesions
Hours		
4	10	6
6	10	6
9	10	17
10	10	15
12	10	15
15	10	2

General conclusions.

The experiments described in this paper point to some interesting facts as regards the spread of the virus by movement from cell to cell. After a leaf has been inoculated on the upper surface, the virus multiplies in the epidermal cells and may be detected in the mesophyll below in as short a period as 4 hours; in the latter tissue, which comprises about 5 layers of palisade and spongy parenchyma, the virus continues its course, and at the end of about 30 hours (or about 36 hours after inoculation), it has passed into the lower epidermis. Since the thickness of the lamina varies from 250 to 300μ near the midrib and about midway between the base and the tip of a well-developed leaf of *Nicotiana sylvestris*, this would indicate that the virus travels at an average rate of approximately 7 to 8μ per hour through a tissue comprising five layers of cells of the mesophyll and one each of the lower and upper epidermis.

This movement at the rate of about 7 to 8μ per hour is so slow that it may well be conceived as taking place by diffusion; but it is difficult to invoke the aid of this mechanism to explain the higher rates of movement of this virus within its hosts. It is probable that, although the virus cannot spread to a considerable distance in a short time by diffusion, this may be an important route by which it travels through the parenchyma to those tissues in which it is mainly carried within the plant.

Summary.

Ordinary tobacco-mosaic virus passes from the upper into the lower epidermal cells in sufficient concentration to be detected 36 to 40 hours after inoculation of a well-developed leaf of *Nicotiana sylvestris*, and continues to increase in concentration for some time.

The time taken by the virus to travel from the epidermis to the mesophyll below is about 4 hours.

Considering the thickness of the leaf of *Nicotiana sylvestris* as 275μ on an average, the rate of spread of the virus in the leaf tissue, under the conditions reported herein, is roughly 7 to 8μ per hour; this rate of movement is independent of the movements of water and food in the leaf.

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THE EFFECT OF DILUTION ON THE THERMAL DEATH RATE OF TOBACCO-MOSAIC VIRUS.

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Plant Pathologist to the Bombay Government.

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It is now generally recognised that the thermal death point is a feature of considerable diagnostic value in the description and differentiation of plant viruses Johnson and Grant [1932] have shown that this property of a virus remains constant under different conditions, and is not influenced by the host species in which the virus may develop. Mckinney [1927] has, however, shown that the thermal death point of a dilute sample of ordinary tobacco-mosaic virus is lower than that of the undiluted juice, and recently Price [1933] has come to a similar conclusion that there is a correlation between the water content of a virus sample and its thermal death point. In his studies Price used a dilution of 1 in 20 of tobacco-mosaic virus, and his results therefore do not indicate whether the thermal death point of the virus will continue to fall progressively for each successive dilution. The experiments described in this paper were designed chiefly to test this view.

MATERIALS AND METHODS.

Ordinary tobacco-mosaic virus (*tobacco virus 1*) was used in these experiments. The diseased tissue of tobacco plants of the variety Connecticut Havanna No. 38 was ground in a mortar, and the juice was pressed out by squeezing the pulp through cheese-cloth. Distilled water was distributed in 9 c. c. volumes in four flasks, to one of which one c. c. of diseased plant juice was added and well shaken. This gave a dilution of 1 in 10. To the second flask was added one c. c. of the 1 in 10 dilution to make a dilution of 1 in 100. In this manner dilutions of 1 in 1,000 and 1 in 10,000 were made up.

The diluted juice to be tested was measured in a pipette, and 2 c. c. of it was placed at the bottom of a very thin-walled test tube ; care was exercised that the juice did not touch the sides of the tube. Each tube was also singed in flame before it was tightly stoppered. The tubes were then immersed almost to the neck in an agitated, electrically heated water bath, in which temperature was controlled within 0·2° C. All exposures were made for 10 minutes ; but an additional period of 15 seconds was allowed in each case to permit the juice in the test tube to reach the temperature of the surrounding water. After each exposure the tubes were rapidly cooled in water.

The local lesion method was employed in determining the inactivation or otherwise of a virus sample. The test plants used were *Nicotiana glutinosa* L. and the F_1 plants of the *N. glutinosa* × *N. tabacum* cross. Both the plants proved equally suitable for this work, although the hybrid has a greater leaf surface for inoculation and can be grown more easily than *Nicotiana glutinosa*.

Inoculation was made by rubbing the surface of a half-leaf with a piece of cheese-cloth soaked in the diluted sample of the virus to be tested, the other half of the leaf being left untreated as a control. In the case of the hybrid inoculum was always applied to three leaves; but in *Nicotiana glutinosa* the growing tip, the small top leaves and the old leaves at the base were removed, leaving five leaves for inoculation. Ordinary tobacco-mosaic virus produces local lesions in both *N. glutinosa* and the hybrid (*N. glutinosa* × *N. tabacum*), but it also becomes systemic in the latter host.

All plants were grown in 4-inch clay pots, and after inoculation were left on a bench in a greenhouse maintained at about 85° F. All apparatus was previously sterilised, and the hands were washed with soap between each inoculation.

Experimental results.

In the first experiment it was decided to test four dilutions of the virus at temperatures of 70, 80, and 90° C. All samples were exposed to these temperatures for 10 minutes and inoculated to three leaves of the hybrid (*N. glutinosa* × *N. tabacum*). A duplicate experiment was made after a week. The results of these tests are given in Table I.

Table I.

Thermal inactivation of ordinary tobacco-mosaic virus at four different dilutions.

Dilution	Experiment 1			Experiment 2			
	70°C.	80°C.	90° C.	70°C.	80°C.	90° C.	Control (unheated)
1 : 10	273*	29	0	604	88	0	531
1 : 100	26	2	0	261	4	0	207
1 : 1,000	15	0	0	5	0	0	40
1 : 10,000	0	0	0	0	0	0	17

* These figures represent the number of lesions produced on three leaves of the hybrid.

It will be seen that as tested by the local lesion method, the virus samples diluted 1 in 10 and 1 in 100 were inactivated at temperatures between 80 and 90°C., that diluted 1 in 1,000 was inactivated at 80°C. or below, whilst the highest dilution of 1 in 10,000 was rendered non-infectious even when heated to 70°C. These results indicated that the thermal death rate of the agent of ordinary tobacco mosaic was influenced by dilution.

The above experiment was repeated with *N. glutinosa* as the test plant. The results are in essential agreement with those obtained with the hybrid, except that in the case of *N. glutinosa* the virus at a dilution of 1 in 10,000 was still active after an exposure for 10 minutes to a temperature of 70°C. (Table II).

TABLE II.

Thermal inactivation of ordinary tobacco-mosaic virus at four different dilutions.

Dilution	Number of lesions			
	70°C.	80°C.	90°C.	Control (unheated)
1 : 10	470*	72	0	491
1 : 100	98	1	0	97
1 : 1,000	12	0	0	36
1 : 10,000	2	0	0	8

* Number of lesions on 5 leaves of *N. glutinosa*.

In order to study further the thermal death rate of the virus, tests were made at temperatures ranging from 72 to 89°C.; the dilution 1 in 10,000 was omitted from these tests since the unheated sample of the virus produced very few lesions by inoculation to leaves.

It will be seen from Table III that the virus at dilutions 1 in 10 and 1 in 1,000 was inactivated at temperatures above 84° and 78°C., respectively; in the case of the dilution 1 in 100, however, the temperature of inactivation was the same as at dilution 1 in 10, although there was only one lesion produced by inoculation with the dilution 1 in 100 heated at 82° and 84°C.

TABLE III.

Thermal inactivation of ordinary tobacco-mosaic virus at different dilutions.

Temperature °C.	Dilution		
	1 : 10	1 : 100	1 : 1,000
72	..	..	9*
74	..	17	9
76	..	37	2
78	..	7	8
80	..	4	0
82	50	1	0
84	23	1	0
86	0	0	..
88	0	..	..
89	0	..	..
Control (unheated)	649	278	69

* Number of lesions on three leaves of the hybrid.

Two further experiments were made using the hybrid and *N. glutinosa* as test plants. The results are summarized in Tables IV and V, and bring out the following points of interest:

(1) Dilution significantly lowers the thermal death point of ordinary tobacco-mosaic virus. At the dilution 1 in 10 the virus is inactivated at a temperature between 86 and 87°C., at 1 in 100 between 82 and 84°C., and at 1 in 1,000 between 77 and 78°C. The results for the dilution 1 in 100 agree with those reported by McKinney [1927] for a similar dilution of tobacco-mosaic virus.

(2) For each successive dilution of 10 times, there is a drop of approximately 4-6°C. in the thermal death point of the virus. The following table shows the effect of dilution on lowering the thermal death point of ordinary tobacco-mosaic virus:

Dilution	Thermal death-point
0	90—92°C.
1 in 10	86—87°C.
1 in 100	82—84°C.
1 in 1,000	77—78°C.
1 in 10,000	70°C. ? (Table I).

(3) The thermal death rate of tobacco-mosaic virus seems to be influenced mainly by the concentration of the plant juice and the number of virus particles in a sample.

TABLE IV.

Thermal inactivation of ordinary tobacco-mosaic virus at different dilutions.

Temperature °C.	Dilution		
	1 : 10	1 : 100	1 : 1,000
77	..	..	1*
78	..	..	0
79	..	..	0
80	..	1	0
81	..	2	..
82	..	2	..
83	..	1	..
84	44	0	..
85	1	0	..
86	1	0	..
87	0	..	..
88	0	..	..
Control (unheated)	1708	948	273

* Number of lesions on three leaves of the hybrid.

TABLE V.

Thermal inactivation of ordinary tobacco-mosaic virus at different dilutions.

Temperature °C.	Dilution		
	1 : 10	1 : 100	1 : 1,000
77	..	..	2*
78	..	..	2
79	..	..	0
80	..	1	0

TABLE V—*contd.*

Thermal inactivation of ordinary tobacco-mosaic virus at different dilutions—contd.

Temperature °C.	Dilution		
	1 : 10	1 : 100	1 : 1,000
81	..	2	..
82	..	1	..
83	..	2	..
84	82	1	..
85	84	0	..
86	48	0	..
87	1	..	..
88	0	..	..
Control (unheated) . .	657	209	77

* Number of lesions on 5 leaves of *N. glutinosa*.

SUMMARY.

The effect of dilution on the thermal death rate of ordinary tobacco-mosaic virus was determined by means of the local lesion method. The virus was inactivated in 10 minutes at a temperature between 86 and 87° C. at a dilution of 1 in 10, between 82 and 84° C. at 1 in 100, and between 77 and 78° C. at 1 in 1,000. The thermal death point of the virus was close to 70°C. at a dilution of 1 in 10,000.

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LODGING OF STRAW AND ITS INHERITANCE IN RICE (*ORYZA SATIVA*)*.

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(With Plates LVIII and LIX.)

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I. Introduction.

The term lodging is applied to a crop which has partly or completely lain over the ground before the harvests, and which may have more or less become entangled. In some cases, the culms may simply lay over without any perceptible bend at the base of the plant, and in other cases, there may be an abrupt bend in the culms at the lower nodes with even crumpling of the internodes. Lodging in cereals, in a broad way, may result from two sets of causes, (1) the interaction of those hereditary and environmental factors which contribute to the development of weak stems and, (2) external forces which exert no influence whatever on the structure of the stems but which cause lodging through mechanical impact such as violent wind rain

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or hailstorm. We are not concerned here with the latter set of causes. Lodging, essentially, is not a problem of poor soils. It comes into consideration only in soils of average and more than average fertility. Apart from the difficulty experienced in harvesting a lodged crop, lodging may markedly affect both the quantity and quality of the produce harvested, the degree of such loss depending on the stage of the crop at which it occurs. Resistance to lodging is ultimately the expression of the interaction of the physiological factors for the growth of the culms with the environment. During the growing period, the plant is able to adjust itself to external conditions and to make to a certain extent the necessary compensation to its surroundings. From this point of view some crops or varieties of crops are more adaptable than others. Production of lodging-resistant varieties still remain an important aim and one with which the majority of cereal breeders are concerned.

II. Review of literature.

The literature dealing with the problem of lodging in cereals of temperate regions as wheat, barley and oats is quite voluminous. The available literature with regard to these cereals deal chiefly with the following aspects of the character: (1) Environmental conditions that influence; (2) Morphological differences of the plants which could prove an index in determining lodging; (3) Mechanical determinations as diameter and breaking strength of the straws; (4) Determinations of the anatomical differences such as thickness of cell-wall, zone of hypodermal or supporting tissues as sclerenchyma; (5) Chemical determinations consisting of the estimation of nitrogen, carbohydrates, inverted sugar and easily hydrolysable carbohydrates, etc.; (6) Varietal adaptations; and (7) Modifications of the cultural practices to avert or minimise damage due to lodging of the crop. The main conclusions arrived at may be summarised as (*a*) that no one factor of the morphological aspect of the problem can be used as an index of standing power, (*b*) that stiffness of straw alone is not an adequate guide to resistance to lodging, as a strong root system is equally important, (*c*) that resistance to bending of the stem is a best measure of standing power, (*d*) that resistance to lodging is dependent not only on the length but also on the elasticity of the culm, the number and extent of the leaves and other characters, (*e*) that lodging is associated with higher average percentage of straw and longer and lighter ears, (*f*) that standing power is associated with less of tillering, (*g*) that lodging is the result of a decrease in the amount of dry matter per unit length of culm, probably dependent on smaller amounts of lignin and other reserve polysaccharides, (*h*) that low content of dry matter is associated with thin-walled cells and a comparatively narrow zone of hypodermal or supporting tissue, and (*i*) that the weakness of the internode is due to a loss of

strength of the mechanical tissue for the efficient production of which potassium is essential.

Because of the elusive nature of the character, and the effect of environmental conditions on the same which are generally beyond the control of the grower, literature dealing with the inheritance of standing power is rather scanty. Biffen and Engledow [1926] state that crosses between forms of *T. vulgare* differing in the standing capacity of their straws showed segregation in the F_2 generation, which, while not sufficiently definite for statistical purposes, was still marked enough to serve as a basis for selection. They also state that there was a great diversity of straw types among the progeny of the crossing of Rivet wheat with any of the English wheats. Nilsson Ehle [1923] and Berg [1926] have observed transgressive variation in straw stiffness in the progenies of crosses, the former in wheat and the latter in barley. Howard and Howard [1912] have been able to successfully produce a hybrid wheat combining the two heritable qualities, stiff straw and a strong root system.

The only published work on this character in rice is by Bhide [1925] who found that there was a thicker band of sclerenchymatous tissue at the periphery of the stem, more numerous fibro-vascular bundles, and layers of small rigid sclerenchymatous cells behind the air cavities in the case of the rigid stems. He also noticed an association between strength of straw and the erectness of the flag leaf.

III. Description of the character in rice.

Rice unlike the cereals dealt with in the literature is grown chiefly under swamp conditions so that the stage at which lodging occurs in the field has an important relationship to final yields. If the soil is too rich in fertility there is excessive vegetative growth and the crop is easily laid by adverse weather conditions as severe wind or rain even before the ear-heads emerge out. In this case the crop is practically lost. If the same lodging occurs later, say, when the crop is in flower, the loss of crop by lodging is chiefly brought about by poor setting of seeds. Should the lodging occur at a still later stage, after the grains are formed, the grains get soaked in water and germinate and the straw also rots or gets mouldy.

Observations were first made to note the nature of lodging wherever it occurred in the pure line collections for two or three seasons. There is a good deal of difference in the type of lodging occurring in the several varieties of rice. There is first the lodging by gradual bending over of the plant with little or no rupture of the tissue. This is of very common occurrence when the soil is over-fertile and a bumper crop is expected. This type of lodging occurs only at the end of the season and

causes no loss of crop unless adverse weather conditions prevail at or just before harvests. The second type of lodging occurs in varieties which have a very open habit of tillering. Here also there is no rupture of the tissue and no damage of crop need be anticipated. The third type of lodging occurs by the whole plant falling over. This is probably due to the defective root system which is superficial and becomes easily displaced. This is more general in varieties of short duration. It also occurs in broadcasted fields. When seedlings are transplanted they are stuck deep into the mud for a few inches, so that the roots developing there provide the necessary anchorage to the growing plant. In the case of directly sown fields, however, the seeds are left on the surface of the soil and a surface root system is the result which is responsible for the plants easily toppling over. The fourth type of lodging which is the most serious, occurs by the rupture of the tissues just a few inches above the ground level. It is possible that this is due in part to the fluctuating water level and the weakening of the supporting tissues consequent on alternate wetting and drying. Where the tissues do not rupture and where the lodging occurs in the earlier stages of the crop the plants make an attempt to recover by "kneeing" at the node and altering the axis of the tiller. As compared to the different types of lodging mentioned above there were some varieties with typically rigid straw with no sign of lodging even at the late stage of the crop.

IV. Observations on lodged and erect Crop in the field.

About a dozen varieties in each of the typically erect and typically lodged groups were marked out and the following observations were recorded for individual plants of either group for three seasons.

Morphological characters.—Length and number of internodes above and below ground level, average panicle length and panicle weight, average girth of straw with and without the enclosing leaf sheath, total height of plant, ratios of floral length (from tip of panicle to the first node at top) to the total height of the plant and to the successive internodes from top, etc.

Anatomical characters.—Thickness of culm, thickness of epidermis, number of vascular bundles, thickness of sclerenchyma of vascular bundles, average dimensions of the vascular bundles, distance of the cortical bundles below epidermis, number and dimensions of the air cells, character of the sclerenchyma band joining cortical bundles when present, etc.

The figures collected have been so variable and not enough for a clear statistical analysis. It is proposed to carry on these studies more elaborately at a future date as time permits. The results have, however, been definite enough to warrant the following general observations being made :—

1. Depth of planting or subsequent sub-aerial growth appear to have little effect on the stability of the crop.

2. There is no definite relation between the length of internodes either above or below ground level and lodging.

3. The lodging lots have a thin or decaying leaf-sheath at or near the water level, whereas the erect lots more frequently have strong and persistant leaf sheaths which may completely shroud the lower internodes and act as an effective support.

4. The diameter of the internodes particularly those just above ground level which are the ones concerned in lodging is definitely greater, with and without the leaf sheath, in the erect than in the lodged lots.

5. Measurements of the ratio of length of floral structure (*i.e.*, above flag leaf) to vegetative portion (*i.e.*, below flag leaf) show that there is no apparent regularity in the errect lots and no connexion with duration of growth. Amongst the lodged lots, the short duration lots are definitely longer in the floral portion, and the long duration lots have greater vegetative length.

6. There is no connection between the weight of the floral portion and lodging.

7. There is no apparent relation between the height of the plant and lodging.

8. The thickness of the culm wall particularly the sclerenchyma portion is more in erect than in lodged lots and this is in agreement with Bhide's observations.

9. The parenchyma portion between the outer row of vascular bundles and the hollow centre is definitely broader in the erect than in the lodged lots.

10. There is no relation between lodging and the number and size of vascular bundles, size of air spaces, thickness of sclerenchyma round and below the vascular bundles. Bhide's findings (with regard to the few layers of small sized sclerenchymatous cells behind the air cavities and connecting the fibro-vascular bundles lying on either side of the air cavities) are found to be true only with respect to certain of the non-lodging varieties. This might be due to his having worked with pure lines of a definite variety of rice, while the results recorded here are with regard to a number of definite varieties.

Observations were recorded for three or four seasons with regard to the morphological characters of a large number of plants taken out of a lodged and non-lodged portions of a field for a few rice varieties in Maruteru Station in Godavari Delta where the problem of lodging is rather acute. In the area represented by this sub-station the soil is a very heavy clay that cracks deeply during the summer months, March-June, after the harvest of the previous rice crop. With a dry summer the soils become rich in nitrates at the time of planting the crop, early June, and the crop invariably lodges in the better kinds of soils even without the application of any fertiliser. The rice growers are usually prepared for this contingency and the local practice in trying to avert or minimise

the damage due to this lodging consists in 'topping' or cutting off of the leafy portions a month or so after transplanting or grazing down the crop with cattle. Numercial measurements of internodes in the lodged and erect portions have definitely established the fact that plants in the lodged portion have longer bottom internodes as compared to plants which do not lodge. The lengths of the lower internodes are definitely smaller in the non-lodged plants when compared to lengths of successive internodes from top. When 'topping' of the plants is resorted to, it is found that the lengths of the lower internode get reduced and this gives more stability to the plant [Srinivasan, 1930]. Though 'topping' has to be practised to prevent premature lodging of the crop, experiments conducted at this station have shown that the cutting should be done judiciously, and fairly early, if it is not to affect the final yield of the crop. Application of artificial fertilisers like ammonium sulphate, which practice is finding favour with the cultivators, early and close planting of the crop, all contribute to make the crop lodge if sufficient precautions are not taken. This naturally takes us to the consideration of the cultural practices that could minimise the loss of crop due to premature lodging.

V. Practical considerations.

The initial high fertility of the fields, early planting, and application of fertilisers are factors contributing to bigger yields. The object of bigger yields is, however, frustrated by the too rank initial growth of the plants under such conditions. Experiments have shown that such rapid initial growth which is indicative of future lodging can be satisfactorily averted by adjusting the cultural practices which consist of a judicious pruning of the vegetative growth early enough, planting the seedlings wider apart where early planting is inevitable, planting seedlings which have been kept for some time after they are pulled out of the seed-bed, and application of the fertiliser in partial doses gradually instead of a single big application. In fields which are over-fertile to prevent the crop from lodging the following practice is also sometimes successfully practised. The seeds of two varieties of rice, one of short duration and the other of long duration, are mixed together in definite proportions and sown together in the seed-bed and transplanted later. At the time the early crop is harvested the long duration crop gets pruned sufficiently and grows later without lodging. Another cultural practice adopted to dodge the problem of lodging is to transplant the crop twice. A month or six weeks after the first transplanting when the plants have started tillering and growing vigorously, the plants are pulled out and retransplanted sometimes separating the tillers if there are already too many in a plant. This practice cannot, however, be adopted for varieties of less than 5 to 6 months in duration.

VI. Inheritance of lodging.

Materials for crossing.

F_2s of two sets of crosses, one made during 1928-29 and the other during 1929-30, for studying rice character revealed segregation for the lodging and erect nature of the straw and the examination of the parents of the crosses gave the following particulars (Plate LVIII).

——	T. 458	T. 63	T. 118
Average height, inches . .	45	67	49
Average flowering duration, days.	112	140	107
Average number of tillers per plant.	4·1	14·3	12·3
Straw character . . .	Very stiff, stout and remain erect after they are quite dead.	Very tall, lanky typically lodging, all culms come down flat on the ground with the breaking at the 1st and 2nd nodes above ground level even before the plants are ripe.	Not tall, but typically lodging with tillers spread on all sides, coming down flat with breaking of the 3rd and 4th nodes above ground level.
Leaf sheath	Thick persistent and closely adhering and covering the respective internode.	Thin and loose and does not encase the internode.	Thin and loose, partly encases the respective internode.
Panicle	Long drooping and bunched, grains, coarse, and big.	Medium, loose and drooping.	Medium, loose and drooping.
Sterility	There is always a certain amount of sterility occurring up to 10—20 per cent.	Normal setting .	Normal setting.

VII. F_1, F_2, and F_3 results.

From the description of the F_1s, it was found that the straw character was not observed in the early stages but it was seen that there was a definite tendency of the F_1 plants for lodging.

On account of the presence of unsetting in one of the parents, the F_1s showed a considerable amount of unsetting, some of the panicles having scarcely half a

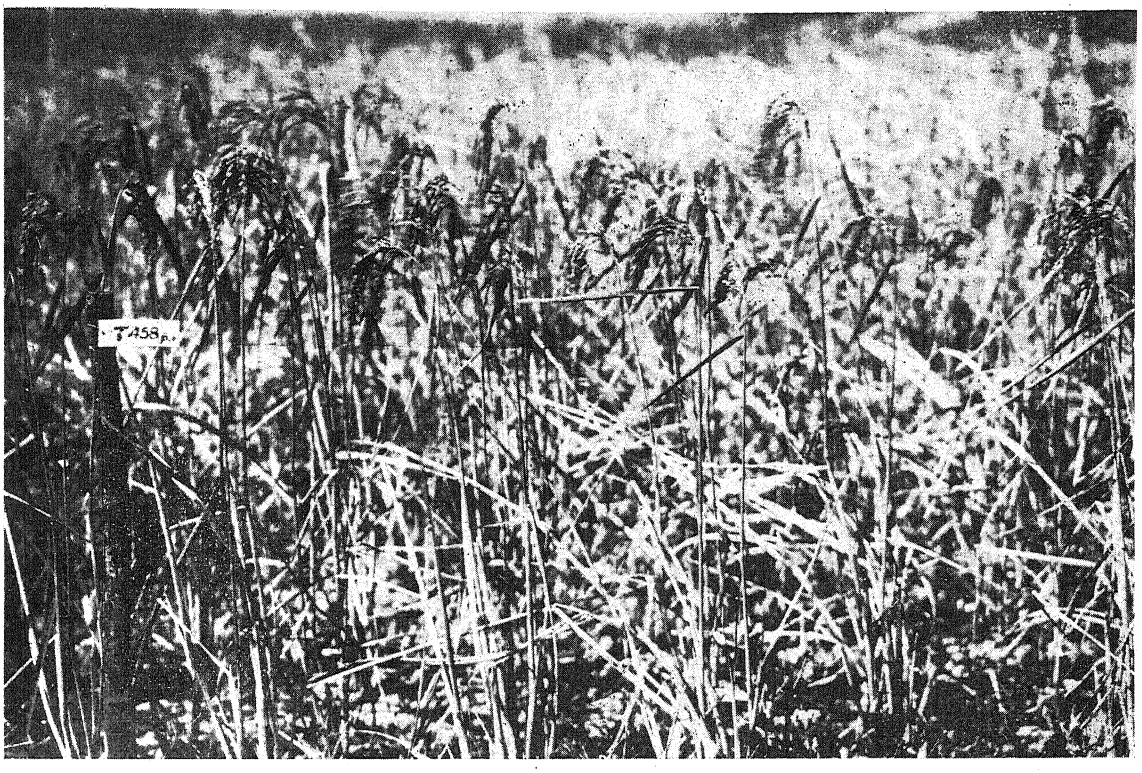
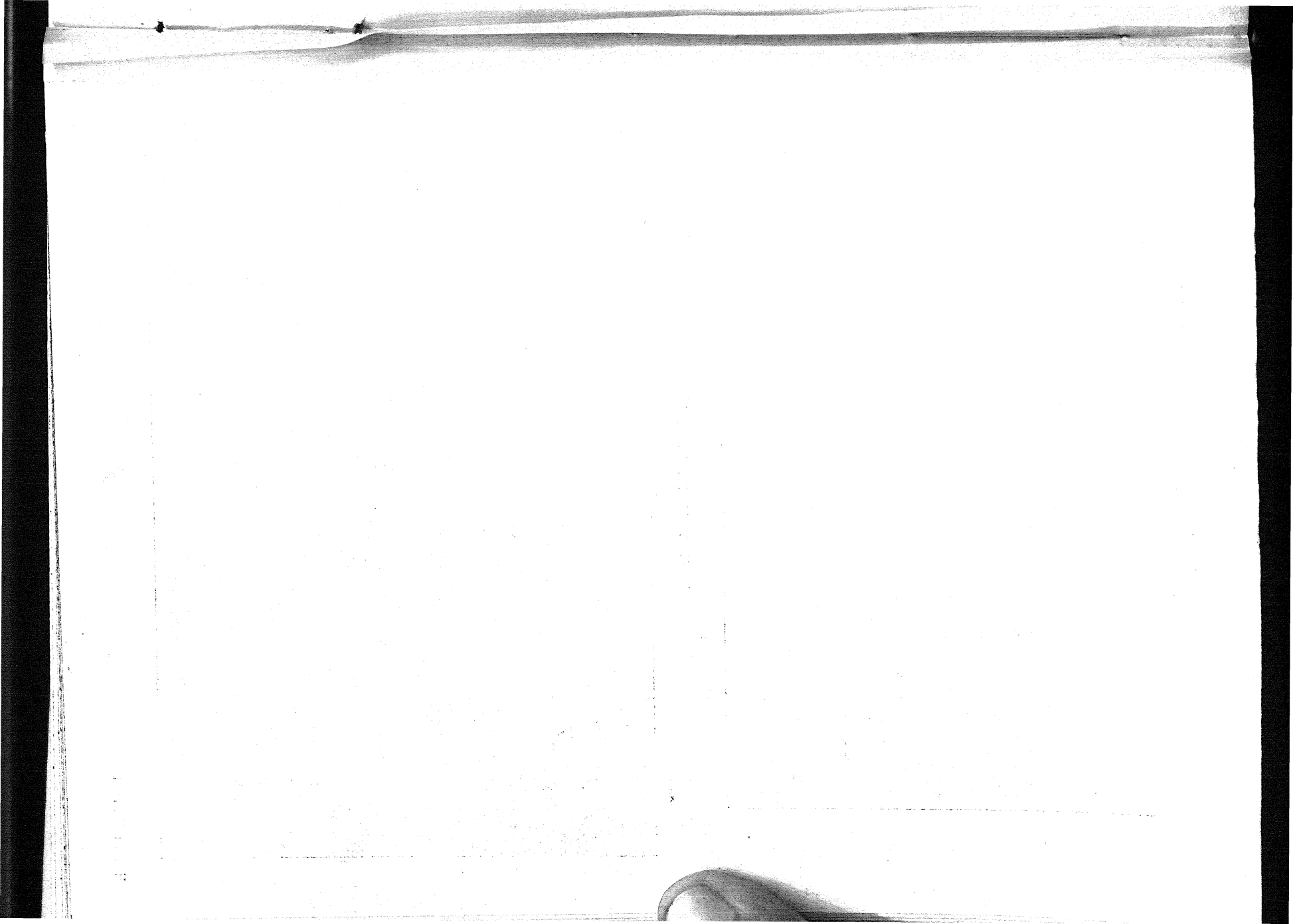


Fig. 1. T. 458—erect straw.



Fig. 2. T. 118—lodging straw.



dozen ripe grains in them. As regards flowering duration the F_1s were nearer the early parent and as regards height they were just like the taller parent as shown below :—

——	T. 63	F_1	T. 458	F_1	T. 118
Average height of plant, inches . .	70	62	50	56	55
Average flowering duration, days . .	133	98	110	92	117

F_2 results.

T. 458 × T. 63.—There was a definite segregation for all the characters examined, plant height, flowering duration, tillering and straw character although it was not easy to put them into definite groups. The best time to examine the plant for straw character has been found to be immediately after the grains are well developed and just before the harvests. Because of the segregation for duration the families had to be examined a number of times and notes taken of individual plants with regard to their straw character. Four groups were adopted in the classification :—(1) those that had definitely gone down with breaking at the 1st or 2nd node above ground level, (2) those with a very open habit of tillering and showing a tendency for the tillers to become prostrate, (3) plants with an open habit of tillering but with no apparent tendency to lodge, and (4) plants with absolutely erect, rigid straws. Evidently there is segregation for the habit of tillering, open or compact, as well as for the lodging and erect nature of the straw.

TABLE I.

Ratios of the 4 groups in the F_2s of T. 458×T. 63.

F_2 Numbers	Groups			
	1	2	3	4
11955	256	98	58	36
11956	171	75	42	49
11957	269	106	18	87
	696	279	118	172
	975		290	
Expected on a 3 : 1 ratio	948		316	

Leaving the habit of the tillers out of consideration, for the lodging character, it would appear reasonable to combine groups 1 and 2 together and 3 and 4 together and this gives apparently a 3 : 1 ratio of lodging to non-lodging.

T. 458×T. 118.—The same character was involved in this cross as well and the F_2s were classified for their straw character as in the previous case, but no attempt was made to separate groups 1 and 2. The ratios obtained in 2 F_2 families are given in Table II (Plate LIX).

TABLE II.

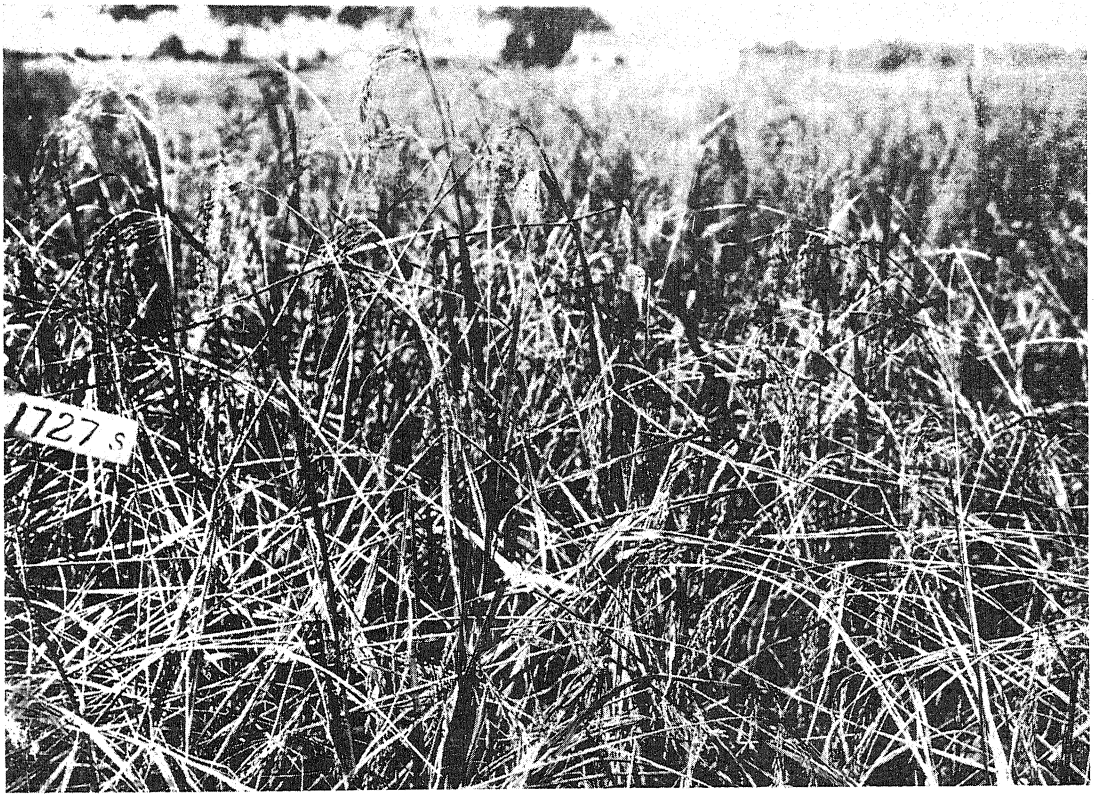
Ratios of the 4 groups in the F_2s of T. 458×T. 118.

Family	Groups		
	1 and 2	3	4
1727	380	40	79
1728	758	116	142
	1138	156	221
	1138	377	
Expected on a 3 : 1 ratio	**1137**	**379**	

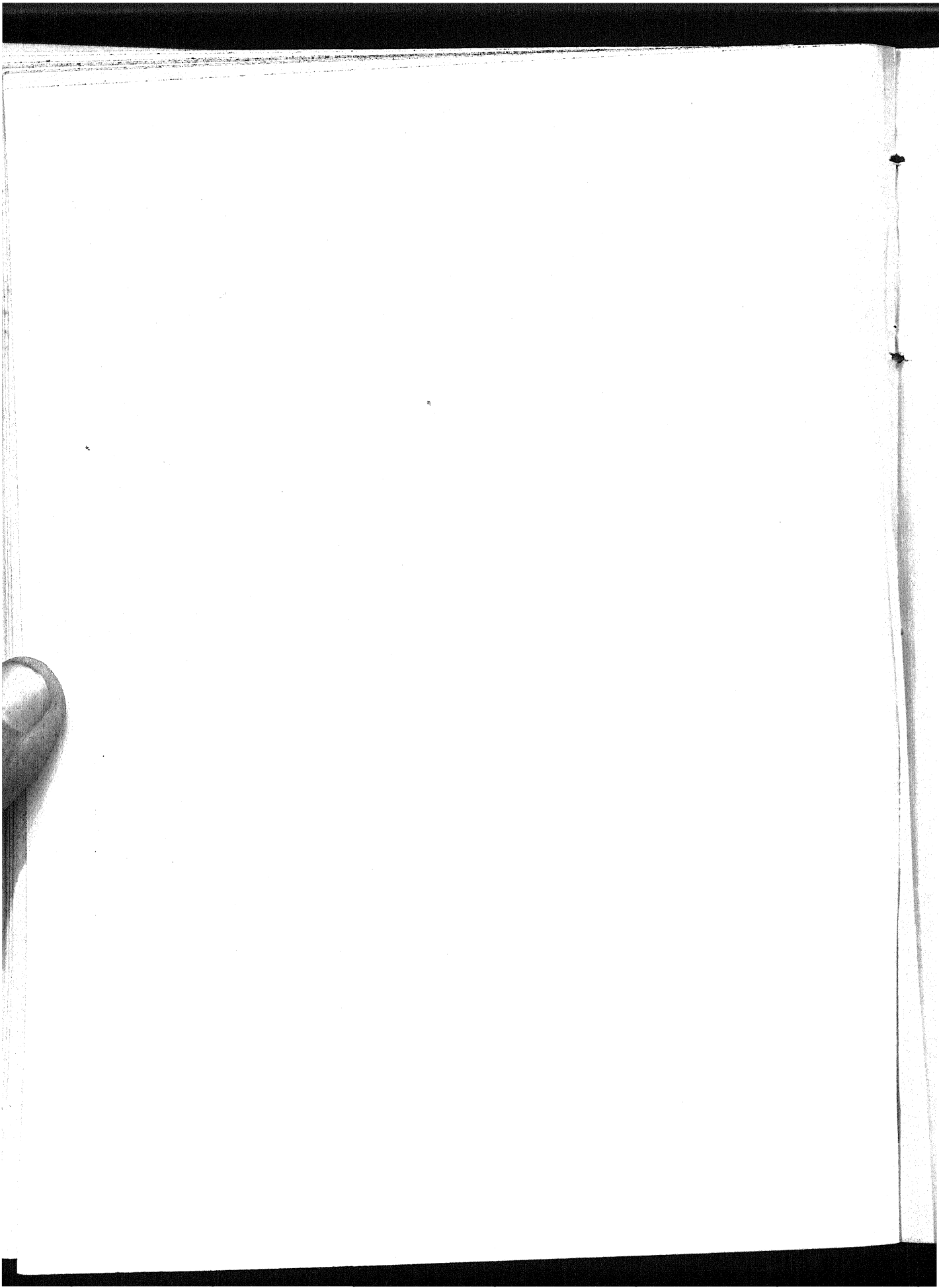
Here also the 3 : 1 ratio of lodging to non-lodging straw is definite. It is surprising that this important character of the straw could behave as if it were due to a single Mendelian factor. The distinction between groups 3 and 4 consisted only in the habit of tillering, the tillers remaining compact in 4, while they were spreading in 3. If this character of tillering had been ignored, naturally group 3 would have to be added on to the first two groups, in which case the ratios become unintelligible except for the fact that the last group 4 forms about 1/7 of the total population in both the crosses.

F_3 results.

To make sure of the F_2 ratios, one family of the cross T. 458 × T. 63, 11957, was carried over completely and grown as F_3. On account of the problem of unsetting mentioned earlier which appeared in varying amounts in the F_2s, several of the F_2 plants had hardly any seed in them and thus the actual number of F_3 families grown was much fewer than the F_2 plants. Since the size of each family had also been restricted for want of space, no detailed observations were recorded for individual plants with regard to the straw character but the families were



F_2 of T. 458 × T. 118 showing both types.



classified into three groups : (1) those that were pure for the lodging character, (2) those that were giving both lodged and non-lodged plants, and (3) those that were pure for the non-lodged nature. The 323 F_3 families grown gave :—

	No. of families.	No. on the basis of a 1 : 2 : 1 ratio
Pure for lodging	71	**80·75**
Giving both types	178	**161·50**
Pure for non-lodging	74	**80·75**

The above is not very different from a 1 : 2 : 1 ratio. Evidently there is only a single-factor difference between the erect and lodged straw character in the crosses studied and it is probable that the open and close habit of tillering of varieties could make it appear complicated, as obviously a plant with a spreading habit of tillering might be mistaken to possess lodging straw. A number of selections made in the two groups, pure lodging and pure non-lodging, have been grown as F_4s and they have been found to breed true for their F_3 character. To make sure whether this straw character is really a simple one, further work is under way in crosses undertaken between the extracted types from the above F_3 family, and between other pure lines where the habit of tillering, flowering duration, spikelet sterility are almost similar in the parents.

VIII. Association between lodging and other characters.

1. Tillering.—Tillering generally is an index of vigour in all cereals and though varieties of rice have been found to differ greatly with regard to this character, the effect of any adverse or favourable environmental condition has been seen first to effect the tillering of the plant. The parents of the two crosses, as was shown earlier, differ widely with regard to their mean number of tillers. The actual number of tillers per plant were counted individually for the F_2s of T. 458 and T. 63 at three stages, the last count being taken soon after the finish of the flowering phase. The F_2 families gave at this last count :—

	Mean number of tillers per plant
T. 458, non-lodging parent	4·4 ± 0·2
T. 63, lodging parent	14·3 ± 0·4
11955 }	9·9 ± 0·2
11956 } F_2s	8·6 ± 0·1
11957 }	7·1 ± 0·1

Though the mean of the whole F_2 is about intermediate between the means of the two parents, the tillering of the four groups classified according to the straw character mentioned in Table I shows a gradual falling off towards the non-lodging type.

Groups	Mean number of tillers per plant	
1	10·9 ± 0·2	Average of the totals of the 3 F_2 families, 11,955—11,957.
2	8·9 ± 0·2	
3	7·1 ± 0·1	
4	5·3 ± 0·2	

The difference in the mean number of tillers per plant is still more apparent when all the plants showing different degrees of lodging (groups 1 to 3) is compared to the typically non-lodging type (group 4) in one of the F_2 families.

Group (1 to 3) combined	7·89 ± 0·11
Group 4	4·00 ± 0·17

A similar result is obtained even in the other cross T. 458 × T. 118 as shown below.

	Mean No. of tillers per plant
T. 458, non-lodging parent	3·8 ± 0·1
T. 118, lodging parent	12·3 ± 0·3
1727 } F_2 families	8·9 ± 0·1
1728 } F_2 families	8·8 ± 0·1

Tillering and straw character.

Lodging (Groups 1 to 3 of Table II)	9·92 ± 0·11	Average of totals for the two F_2 families 1727 and 1728.
Non-lodging (Group 4 of Table II)	5·09 ± 0·20	

The number of tillers per plant were also counted for the pure lodging and pure non-lodging families in F_3 family, 11957, and their frequencies are given in Table III.

TABLE III.

Functional tillers in lodging and non-lodging families in the F_3 progenies of T. 458 × T. 63.

Mean number of tillers per plant of F_3 families	Frequencies		
	Pure for lodging	Splitting families	Pure for non-lodging
2	..	..	1
3	1	7	5
4	4	35	26
5	3	35	18
6	10	31	8
7	12	34	6
8	16	14	1
9	12	5	1
10	6	2	..
11	2	0	..
12	3	2	..
13	2	..	..
14	1	..	..
Means	7·9 ± 0·3	5·9 ± 0·1	4·8 ± 0·2

It is therefore very definitely clear from the above that the straw character is genetically associated with such an important economic character as tillering, the non-lodging plants having a fewer number of tillers per plant than the lodging plants.

2. Flowering duration.—The flowering duration in rice has been proved to be a Mendelian character, sometimes simple and at other times complicated according to the number of factors involved [Ramiah, 1933]. The mean flowering duration of the F_2s of the two crosses are given below first separately and later according to their straw character.

	Mean flowering duration in days
T. 458, Non-lodging parent	114·5 ± 0·2
T. 63, Lodging parent	140·2 ± 0·1
11,955	108·9 ± 0·3
11,956 F_2s	110·3 ± 0·3
11,957	111·2 ± 0·3
T. 458, Non-lodging parent	109·7 ± 0·3
T. 118 Lodging parent	107·5 ± 0·1
1,727	95·3 ± 0·3
1,728 F_2s	98·8 ± 0·2

Mean flowering duration in days	Character of straw	
	Various degrees of lodging	Non-lodging
Totals for 3 F_2s of T. 458 × T. 63	109·4 ± 0·2	115·4 ± 0·5
Totals for 2 F_2s of T. 458 × T. 118	96·5 ± 0·2	100·2 ± 0·5

Though the F_2 means are even earlier than the parent concerned, when the straw character is taken into consideration the groups, lodging and non-lodging, are found to have definitely different mean durations showing the association between straw character and duration. The test of independence in a $2 \times n$ classification (Fisher) applied to this pair of characters gives a very insignificant value, $P = \cdot 000$. The mean duration of the two groups, pure lodging and pure non-lodging, of the F_2s were 92·9 ± 0·48 and 105·7 ± 0·88 respectively which again shows definite association, the non-lodging plants being definitely later in duration than the lodging plants.

3. Plant height.—There was a big difference in height between the parents in the cross T. 458 × T. 63 and very little in the cross T. 458 × T. 118. The mean heights of the F_2s independently and in relation to nature of straw are given below :—

—	Mean height in inches.
T.458, Non-lodging parent	44·3 ± 0·7
T.63, Lodging parent	67·5 ± 0·4
11955 } F_2s {	52·7 ± 0·2
11956 } F_2s {	53·1 ± 0·2
11957 } F_2s {	50·0 ± 0·2
T.458, Non-lodging parent	46·5 ± 0·3
T.118, Lodging parent	48·6 ± 0·2
1727 } F_2 {	47·2 ± 0·5
1728 } F_2 {	46·4 ± 0·2

	Mean height in inches	
	Character of straw	
	Various degrees of lodging	Various degrees of non-lodging
Totals for 3 F_2s of T.458 × T.63	51·0 ± 0·16	50·8 ± 0·8
Totals for 2 F_2s of T.458 × T.118	47·0 ± 0·10	44·7 ± 0·4

The tabulations show that there is no apparent relationship between plant height and nature of straw.

4. Floret sterility.—While the parents of the cross T. 458 and T. 63 were almost fertile exhibiting only about 30 and 14 per cent. respectively of floret sterility, the F_2 plants covered a wide range from almost complete fertility to almost complete sterility indicating the interaction of multiple factors controlling floret sterility. When grouped in relation to the straw characters, lodging and non-lodging, it was found that there was practically no association between sterility and straw character.

		Floret sterility (percentage)
Parents	T.458	30 ± 1·5
	T.63	14 ± 1·3
F_2 11957	General mean	60·7 ± 1·3
	Lodging groups mean	59 ± 1·3
	Non-lodging groups mean	63 ± 2·6

IX. Summary.

1. Examination of the pure lines of rice varieties show that the straw character varies considerably and varieties can be grouped into lodging and non-lodging types.

2. Even in the lodging groups there are different types of lodging although it is not possible to classify them except in the earlier stages.

3. The morphological studies on the plant show that there is no single factor which could be used as an index of the lodging nature of straw. The only consistent result is that non-lodging varieties have thick, persistent leaf sheaths tightly encasing the bottom internodes and these internodes have comparatively a bigger diameter.

4. Apart from varietal differences the conditions which bring about lodging of a crop in the field are high initial fertility, early and close planting and applications of nitrogenous fertilisers. Damage due to lodging of the crop can be averted or minimised by suitably modifying the cultural practices as judicious pruning of the crop in the early stages, wider planting where early planting is inevitable, double transplanting and growing a mixed crop of a short and a long duration variety.

5. From the study of crosses between two typically lodging varieties and a typically non-lodging variety of rice, it is seen that there is probably only a single-factor difference between the two.

6. There is genetic association between the character of the straw on one hand and such characters as tillering and duration on the other. Non-lodging nature of straw is linked up with poorer tillering and longer duration. There is apparently no relation between plant height and straw character, nor between straw character and floret sterility.

ACKNOWLEDGMENTS.

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OAT SMUT IN INDIA.

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(With Plate LX)

The first specific mention of oat smut seems to have been made by Tragus in 1552 when he called it Ustilago because of the burned and charred appearance of the panicle. Lobelius in 1591 also referred to it and made a sketch of it under the name *Ustilago avenae*. Linneaus' reference to the smuts is interesting in that he placed them in the group *Chaos ustilago* or *Reticularia ustilago*. He first thought of them as infusoria but seems to have recognised them later as plants. Bulliard in 1791 classified all the smuts of cereals under the name *Reticularia segetum* and this was the first time that they were considered as fungi. Persoon in "Synopsis Methodica Fungorum" published in 1801 also recognised the smuts as fungi and gave the name *Uredo segetum* var. *tritici*, *hordei* and *avenae* for the forms on wheat, barley and oats respectively, but Ditmar in 1813 called the oat smut *Ustilago segetum*. It was not until 1888 when Jensen after considerable infection experiments had clarified the position and demonstrated that wheat, barley and oats had each their respective and specific smut pathogenes that the loose smut of oats come to have the name by which we know it to-day, *Ustilago avenae* (Persoon) Jensen. He did not at that time distinguish however between the two smuts on oats, the loose and covered smuts.

The distinction of recognising the two smuts of oats belongs to Kellerman and Swingle who observed the two forms in Kansas in 1890. They considered the pathogene of the newly discovered smut as a variety of the loose smut fungus and called it *U. avenae* var. *levis*. In 1893 Wille named this same fungus *U. kolleri* and Magnus, independently in 1896, raised Kellerman and Swingle's varietal name to specific rank as *U. levis* (Kellerman *et* Swingle) Magnus. According to the Plant

Pathology Sub-committee of the British Mycological Society [1929] who went carefully into the question of nomenclature and priority, the name of the covered smut fungus of oats is *Ustilago kolleri* Wille.

Both these smuts of oats occur in India. In Butler's "Fungi and Disease in Plants" [1918] a coloured drawing of the covered smut is given in figure 60 but the description of the smut on pages 179-182 is of loose smut. This discrepancy between the figure and the description led the writer to examine the material in the Pusa Mycological Herbarium and an interesting discovery was made that the common oat smut in India is due to *U. kolleri* (=*U. levis*). Butler and Bisby [1931] in their "Fungi of India" do not even list this fungus.

In the field the two smuts can be easily distinguished. The spores of both the fungi are produced in the spikelets and these are dusty brown in the case of loose smut (*U. avenae*) and black brown in the case of covered smut (*U. kolleri*). The real macroscopic distinction is however in the fact that in the case of loose or naked smut all the floral parts are completely destroyed while in the case of covered smut, the spores are concealed by the glumes which remain intact.

Under the microscope it is observed that the spores of *U. avenae* and *U. kolleri* are of the same size, 5 to 9μ, the longest being 11μ, but the surface of the exospore of the loose smut fungus, *U. avenae*, is minutely echinulate while that of covered smut, *U. kolleri* (=*U. levis*) is smooth and this is the fundamental distinguishing feature. The photograph and the camera lucida drawing in Plate LX will make this point clear.

The earliest collection of oat smuts in India was made by Sir George Watt at Dehra Dun in March 1893 and specimens of collections made since then by Burkill Cave, Butler, Mitra, Das, Merriman and Jiwan Singh are represented in the herbarium. A majority of these specimens are of covered smut but all of them bear the name *U. avenae*. That many of these should be named *U. kolleri* became clear after a thorough inspection.

The specimens were first examined with a hand lens to see that the glumes were intact and this was supplemented by an examination, under the microscope, of the spores. Small quantities of these spores were soaked on the slide in a drop of Amann's Lacto-phenol mounting medium and they were then examined under the oil immersion lens. The chlamydospores of *U. avenae* distinctly showed very fine spines on the surface but the surface of the exospore of *U. kolleri* was entirely smooth. The results of the examination are recorded in Table I.





Loose and covered smuts of oats together with their respective chlamydospores. (×1440.)

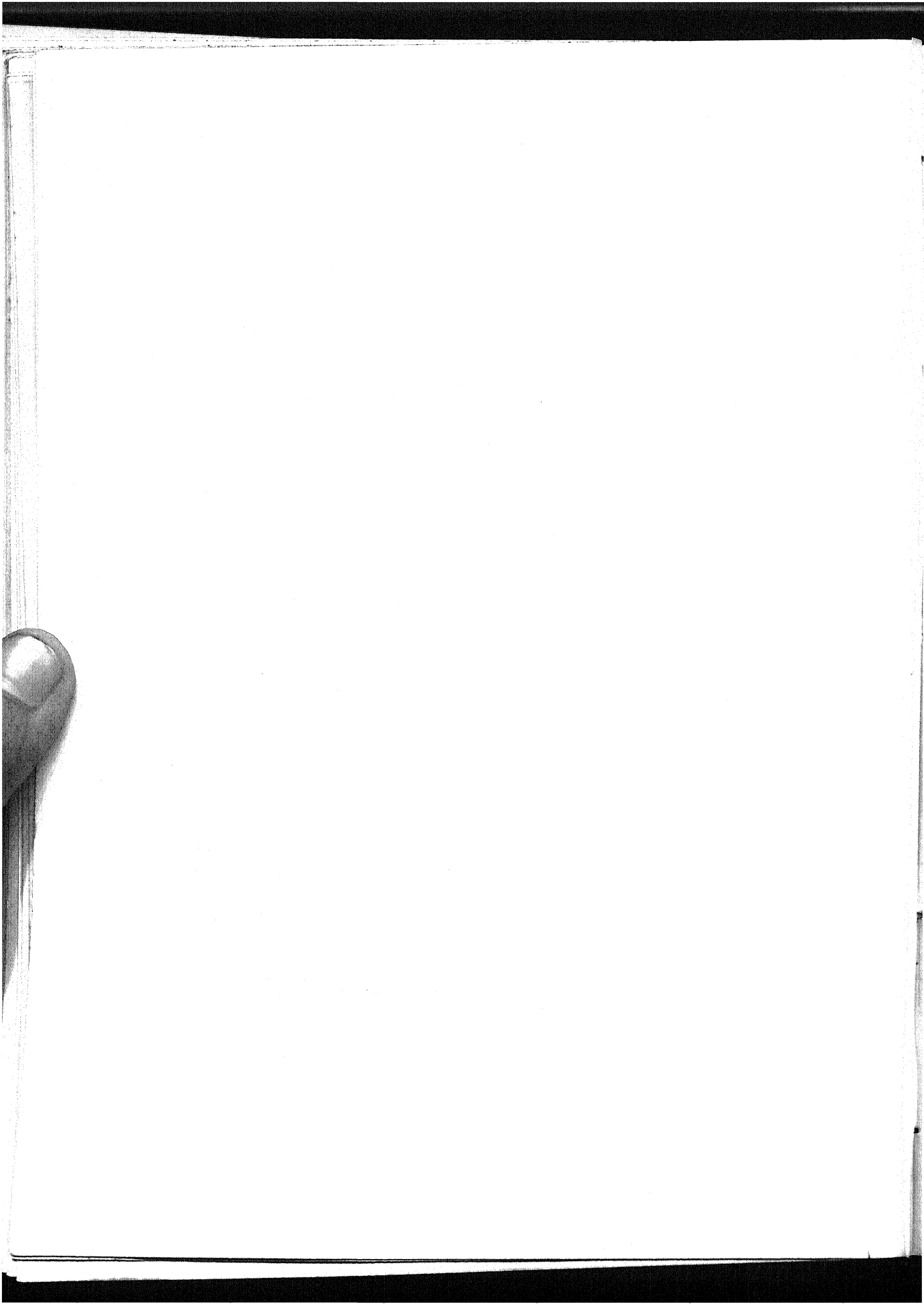


TABLE I.

Herbarium specimens of oat smuts and their nomenclature.

Name on specimen	Locality	Date	Collector	Result of examination
U. avenae . .	Dehra Dun .	1893	Watt . .	*U. kolleri.*
Do. . .	Dumraon .	12th March 1899	Do. . .	*Do.*
Do. . .	Kainka near Delhi.	24th February 1902.	Burkill . .	*Do.*
Do. . .	Jagannathpur .	29th April 1903	Cave . .	Both smuts are present.
Do. . .	Dehra Dun .	2nd March 1903	Butler . .	*U. kolleri.*
Do. . .	Pusa . .	26th March 1907	..	*Do.*
Do. . .	Pusa . .	13th February 1911.	..	*Do.*
Do. . .	Lahore . .	March 1911 .	Das . .	*Do.*
Do. . .	Landhi, Sind .	28th February 1916.	..	*Do.*
Do. . .	Pusa . .	23rd February 1917.	Taslim . .	*Do.*
Do. . .	Lahore . .	14th March 1917	Mitra, S. . .	*Do. and U. avenae.*
Do. . .	Motihari . .	11th March 1920	Merriman .	*U. kolleri.*
Do. . .	Pusa . .	19th February 1925.	Mitra, S. . .	*Do.*
Do. . .	Amritsar . .	1931	Jiwan Singh .	*Do.*

Sydow and Butler [1906] examined the material collected in 1903 at Dehra Dun and at Jagannathpur and bearing the herbarium number 458 and determined the fungus as *U. avenae* in their " Fungi Indiæ Orientalis, Part I ". The Jagannathpur collection on examination now showed the presence of both the fungi, *U. avenae* and *U. kolleri*, and it is possible that the specimen that was examined at that time by them from this collection was that of loose smut. The Dehra Dun collection of 2nd March 1903 consists at present of a single panicle which is that of *U. kolleri*. In the collection made at Lahore by S. N. Mitra in 1917 panicles of both loose and covered smut are present. Clinton [1904], MacAlpine [1910], and Butler [1918], mention the fact that the chlamydospores of *U. avenae* are lighter coloured on one side than the other. Few spores of the Lahore collection and not all showed this characteristic.

In describing the smutted panicle of oats, Butler [1918] on page 179 of his book states that the wall of the ovary persists as a distinct envelope around the spore mass and he evidently considered the naked or loose smut condition as a further stage in the development of the covered smut. He mentions the spiny character

of the exospore so that he certainly examined the chlamydospores of *U. avenae* but apparently the smooth-walled spores of *U. kolleri* were missed.

Clinton [1904] expressly mentions that these species occur more or less mixed in exsiccati. A specimen distributed under the name *U. avenae* and bearing number 1501 by G. H. Cunningham is really *U. kolleri* and another distributed as *U. levis* under No. 1495 is really *U. avenae*. Even the illustration in Plate VIII of MacAlpine's book [1910] bears a resemblance to a covered smut rather than to a typical loose smut.

In the collections made so far in India the covered smut specimens predominate and it looks as though this smut is the more common smut. An effort will be made to determine this during the next oat season. Heald [1919] is of the opinion that the distribution of the two smuts is controlled by temperature differences. The presence of loose smut in the Lahore collection and its absence in the collections made at Pusa where the minimum temperatures during winter are not so low does point toward such a general conclusion but further study alone can show whether this is a fact.

Summary.

1. An examination of herbarium specimens of oats smuts at Pusa revealed that the majority of them are of *Ustilago kolleri* Wille and not of *Ustilago avenae* (Pers.) Jensen as stated on them.

2. *U. avenae* does occur in India having been collected at Jagannathpur and at Lahore in 1903 and 1917 respectively.

3. Covered smut due to *U. kolleri* seems to be more prevalent than loose smut due to *U. avenae* in India.

4. A survey of the two smuts is being made to determine the relative preponderance of the one or the other.

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A DRY SPRAY METHOD OF TREATING OAT SEED AGAINST COVERED SMUT.

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(With Plate LXI)

In a previous paper the senior author [Mundkur, 1934] has shown that the predominant smut of oats in India, so far as the present information goes, is the covered smut due to *Ustilago kolleri* Wille (= *U. levis*). An effective method of treating the oat seed against this smut is reported in this paper.

The cultivation of oats is restricted at the present time in India to the vicinity of towns and cities with cantonments where the crop is used as forage for horses and mules. The grain of the Indian oats is of rather poor quality and difficult to husk and Shaw and Bose [1933] mention this as a chief reason for the lack of interest in this very nutritious cereal. The cultivation of this crop, according to the same writers, is on the increase and with the introduction of good types which the Botanical Section at Pusa is developing, it is likely to be more popular.

The crop is host to several fungous diseases but in India the greatest damage is caused by the smut disease. Exact figures assessing the loss are not available but Butler [1918] mentions that in certain oat growing sections, about one-tenth of the crop is usually lost due to this disease. In 1904-1905 he found that one plant in every ten was affected by the smut at Dehra Dun and from the writers' experience for the last three years at Pusa where the crop was under observation it became apparent that the disease was causing much damage to the crop.

LITERATURE REVIEW.

That some of the smut diseases were seed-borne seems to have been known even as early as 1755 for Tillet according to de Bary [1853] treated smutted seed grain with lime and reduced the smut in his fields. In 1807 Prevost demonstrated the superiority of copper sulphate as a seed treatment and showed that while one plant out of three was smutted in untreated plots, only one in four thousand was smutted

in treated fields. Since that time several methods are in vogue in countries where oats are extensively grown to treat the seed against smut, predominant among these being the formaldehyde and the organic mercury compound treatments.

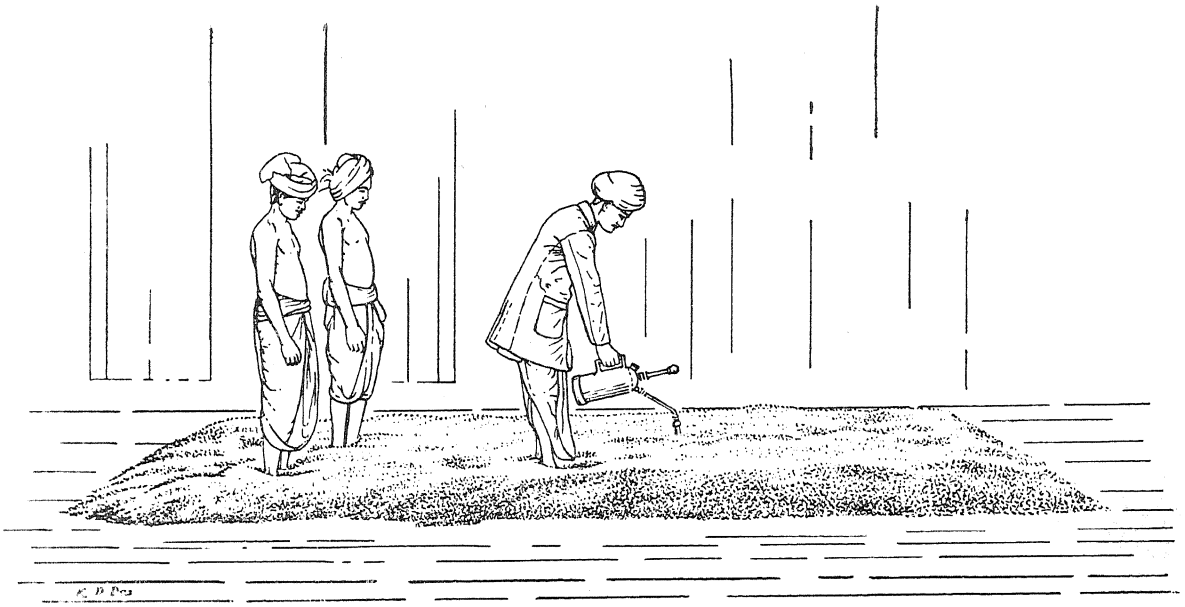
In India the sprinkle method of applying formaldehyde or the copper sulphate solution are some times used whenever smut infestation of seed is very bad. Both these methods are described by Butler [1918] and they have proven of value in reducing smut to a great extent. In the sprinkle method a pound of formaldehyde is diluted with forty gallons of water and the mixture is sprinkled over thirty maunds (one maund=82 lbs.) of grain. The moistened seed is kept covered under sacks for four hours after which it is dried when it is ready for the seed drills. The method is not however popular both because of the large quantities of water that are necessary and the difficulty involved in drying the seed after the treatment.

Because of these handicaps of the wet method of treatment, the writers thought it worthwhile trying the dry spray method of treating the oat seed which is in common use in the United States of America. The method was first suggested by Haskell [1917] and the ease of application of the formaldehyde and the absence of any wetting of seed, have made it popular with oat growers.

Materials and methods.

About one hundred and fifty maunds of oat seed were available for conducting the trials. The seed came from a crop which in the previous year had suffered heavily from covered smut. All of it belonged to a single type B. S. I. About one hundred acres of land were available on the Pusa Farm and about thirty acres were available in the New Area. On the Pusa Farm area ninety-six acres were sown with treated seed and the rest with untreated seed while all the seed for the land available at the New Area was untreated. In addition to this a small plot was also available in the Mycology Section fields where a trial on a smaller scale was conducted.

The method.—The method of applying formaldehyde as originally devised by Haskell had to be slightly modified to meet local conditions. As finally developed the method is as follows : Spread twenty-four maunds of the seed on a clean floor in an even layer which should not be more than eight inches thick. Place a pound of formaldehyde in a six-pint hand pressure-sprayer and dilute it by pouring into the sprayer an equal volume of water. Close the tap and pump the sprayer to obtain proper pressure. One labourer applies the mixture to the grain holding the sprayer close to the seed and walking in the grain in the meanwhile. Two other labourers follow this man turning the seed upside down with their legs and this operation is continued until most of the mixture has been sprayed (Plate LXI).



Method of applying the formaldehyde to oat grain by the dry spray method.

New gunny sacks enough to cover the oat seed when made into a pile are also sprayed with a little of the mixture. The seed is then made into a heap and covered with the treated sacks. The process is simple and takes not more than half an hour in all and is best done on a still evening. The place where the seed is spread should be protected from drafts. The labourers have to be provided with handkerchiefs to cover their nostrils and mouths and they should throw back their faces so as to minimise the chances of their being injured by the formaldehyde fumes. The next morning the heaps are uncovered and the seed is ready for the seed drills.

The seed is hardly wetted by this process and if it cannot be immediately sown it can be stored for some weeks without injuring its viability.

Results.

Pusa Farm and New Area.—Sowing was done in the second and third weeks of October 1933. Four acres on the Pusa Farm were also sown with untreated seed. Germination in all the plots was quite good but a laboratory test was also carried out to determine the per cent. germination of treated and untreated seed. The results recorded in Table I were carried out in Petri dishes containing moistened filter paper.

Table I.

Germination tests with formaldehyde treated and untreated oat seed in Petri dishes.

Test No.	Treated or untreated	No. of seed per dish	No. germinated	Mean germination
1	Treated	100	97	97·6 per cent.
2	Treated	100	97	
3	Treated	100	99	
4	Treated	100	96	
5	Treated	100	99	
6	Untreated	100	98	96·8 per cent.
7	Untreated	100	98	
8	Untreated	100	95	
9	Untreated	100	96	
10	Untreated	100	97	

From the data recorded in Table I it will be noted that germination in the treated lot of seed was actually higher though there was not any significance in the difference. The treatment had not therefore impaired the germination capacity of the seed in any way.

The crop on the Pusa Farm and the New Area was harvested in March 1934. All the fields where treated seed had been used were carefully examined for

any smutted heads. In an area covering ninety-six acres, there was not a single smutted plant but in the New Area where untreated seed had been sown smut appeared to be rather bad. Even in the four acres on the Pusa Farm where untreated seed had been sown, intensity of smut infection seemed to be severe.

In order to determine the extent of damage actual counts of healthy and diseased plants were made. From one field about thirty-six acres in extent which was sown with treated seed on the Pusa Farm, twenty harvested bundles were selected at random and a like number was also brought from the New Area (twenty-five acres) where untreated seed had been sown. Diseased plants were first separated from each bundle and the healthy and diseased plants then counted. The results are recorded in Table II.

TABLE II.

Incidence of covered smut in oats treated and untreated with formaldehyde by the dry spray method.

Bundle No.	Treated		Untreated		
	Total plants	Plants smutted	Total plants	Plants smutted	Per cent. smut
1	2700	*Nil.*	2317	36	1·55
2	2150	*Nil.*	2344	104	4·43
3	2317	*Nil.*	4266	57	1·34
4	1532	*Nil.*	1613	65	4·03
5	2197	*Nil.*	2221	117	5·27
6	2587	*Nil.*	2956	93	3·15
7	3541	*Nil.*	2804	153	5·46
8	1434	*Nil.*	2392	149	6·23
9	1211	*Nil.*	2309	58	2·51
10	1649	*Nil.*	2574	189	7·34
11	1807	*Nil.*	2577	60	2·33
12	1854	*Nil.*	2632	445	16·91
13	1627	*Nil.*	2672	95	3·56
14	1848	*Nil.*	2819	51	1·81
15	1705	*Nil.*	2654	133	5·03
16	1867	*Nil.*	2024	82	3·09
17	2073	*Nil.*	1717	47	2·74
18	1623	*Nil.*	1637	85	5·19
19	2139	*Nil.*	2413	39	1·62
20	1609	*Nil.*	2235	59	2·64

It will be noted that there was complete control of smut in the treated samples while in the untreated samples intensity of smut varied from 1·34 to 16·91 per cent. average being 4·31 per cent. In the four acres on the Pusa Farm where untreated seed had been sown there was also considerable smut but as the crop formed part of

an experiment of the Imperial Agriculturist, no samples could be taken for the purpose of making the counts.

In order to determine the efficacy of the treatment another trial was conducted at the same time in the Mycology Section plots. The seed came from the same source as before and was treated as follows :—

A. Naturally infected seed.

B. Naturally infected seed but treated with formaldehyde by the dry spray method.

C. Same seed as in A but artificially infested with smut spores and then treated with formaldehyde.

D. Same seed as in C but without treatment.

The available land was divided into twenty plots of four lines each, distance between lines being nine inches and each line being sixty-two feet long. The plan of sowing the seed treated in four different ways was arrived at by randomising the plots, the plots being grouped into five blocks of four plots each. Sowing was done on the 16th of October 1933, seed being six inches apart and there being two seeds per hill. Germination was good and there was not any other disease in the crop.

On the 22nd of March 1934 the crop was harvested by uprooting the plants. The total number of plants in each plot and the number smutted were counted, after which the bundles were threshed and the yield of each plot was noted down. The data of germination, plants smutted and yields are given in Table III.

TABLE III.

Germination, extent of smut and yield of formaldehyde treated oat seed, in five blocks of four plots each.

Blocks	Treatments											
	A			B			C			D		
	Germination	Smutted	Yield in lbs.	Germination	Smutted	Yield in lbs.	Germination	Smutted	Yield in lbs.	Germination	Smutted	Yield in lbs.
I . .	703	28	14·7	732	0	16·0	700	0	14·5	717	20	13·5
II . .	732	36	12·6	710	0	15·6	660	0	16·5	734	37	13·7
III . .	722	33	10·2	747	0	10·6	725	0	14·8	758	33	11·6
IV . .	675	39	10·8	736	0	13·0	696	0	15·0	701	28	9·5
V . .	674	34	12·5	721	0	16·5	773	0	14·8	757	46	13·3

In each plot nine hundred and ninety-two seeds had been sown and there should have been that many plants in each plot. As the actual number of plants per plot varied an analysis of variance test was applied to see whether there was any significance in the difference in germination in the variously treated plots. Data are recorded in Table IV.

TABLE IV.

Analysis of variance to determine if the treated plots are significantly worse than the untreated plots in their germination.

Due to	Degrees of freedom	Mean square	
Blocks	4	930·45	
Treatments	3	1165·30	$v_1 = 1165{\cdot}3$
Errors	12	770·00	$v_2 = 770{\cdot}0$
Total	19		

$$x = \frac{1165{\cdot}3}{770{\cdot}0} = 1{\cdot}5133.$$

Referring to Mahalanobis' table [1933] it is found that for $n_1 = 3$ and $n_2 = 12$, the value of x must be 3·49 for five per cent. level of significance or 5·953 for one per cent. level of significance in order that the differences may be considered statistically significant. As the value obtained is very low, there is not any significance so that germination in treated and untreated plots is alike.

As for the incidence of smut, none of the treated plots had any smut.

As for yields, an analysis of variance is given in Table V.

TABLE V.

Analysis of variance to determine whether treated plots yield significantly higher than untreated ones.

Due to	Degrees of freedom	Mean square
Blocks	4	7·751
Treatments	3	$10{\cdot}8618 = v_1$
Errors	12	$1{\cdot}7897 = v_2$
Total	19	

$$x = \frac{10{\cdot}8618}{1{\cdot}7897} = 6{\cdot}069$$

Again referring to Mahalanobis' [1933] table, it is found that for $n_1 = 3$ and $n_2 = 12$, the value of x must be 3·490 at five per cent. level of significance and 5·953 at one per cent. level of significance. The value obtained is more than both these

values showing that the higher yields given by treated plots are statistically significant.

Discussion.

The results given in the foregoing pages have shown that the application of formaldehyde in the form of a concentrated dry spray does not injure the viability of the seed, that smut is completely controlled, and that treated seed yields higher than untreated seed. About twenty per cent. more grain was obtained by subjecting the seed to this treatment. A pound of formaldehyde is enough to treat twenty-four maunds of seed which can be sown on about 25 acres of land. The cost of a pound of commercial formaldehyde is about a rupee at the present time so that the cost per acre works at about eight pies. The cost of labour is negligible. A hand pressure sprayer of six-pint capacity costs about thirty-five rupees which is the only costly item. But one should last several years.

Summary.

Concentrated formaldehyde was sprayed on smutty oat seed with a view to control the smut disease due to *Ustilago kolleri* Wille at the rate of a pound of formaldehyde to twenty-four maunds of oat grain.

In ninety-six acres of oat fields that were sown with treated seed, not a single smutted plant was obtained while smut was very bad in fields sown with untreated seed.

It was noted that treating the seed with concentrated formaldehyde was not injurious to the seed and that treated seed yielded about twenty per cent. higher quantity of grain than untreated seed and that this difference in yields was statistically significant.

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ESTIMATION OF THE NUMBER OF FIBRES ON A COTTON SEED BY DIFFERENT METHODS: A COMPARISON.

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The average number of fibres that are produced on the surface of a cotten seed may be estimated by any of the following methods :—

(A) By the method employed by Turner [1929], where the variations in the length and weight of the fibres that make up the sample are taken into account,*

(B) By dividing the lint weight per seed by the product of the mean fibre length and the mean fibre weight per unit length, got by the ordinary cutting method,

(C) By dividing the lint weight per seed by the unit fibre weight got by weighing whole fibres, as described by Nazir Ahmed [1931] and

(D) By dividing the lint weight per seed by the mean unit fibre weight obtained in Turner's method. It is evident that this method is not independent, as it depends on the method (A), for the value of the unit fibre weight.

No attempt has so far been made to compare the results obtained by the different methods, except that of the writer and Turner [1930], where the values got by the methods (A) and (B) are compared. Even this comparison is not accurate, as the determinations according to both the methods are not made on the same samples. This want of comparative values has led to some misconceptions, an instance of which is the remark of Barritt [1933], where he states that differences observed between the figures given by Ayyar and Ayyangar [1933] and those derived by him are due to "carelessness" of arithmetical errors of these authors. It is proposed to show here that such differences may be entirely due to the variations in the experimental methods employed.

The results recorded in this work are those obtained from nine samples of Co.2 (*G. hirsutum*) and seven of Karunganni 546 (*G. indicum*), each sample representing a single position of the seed in the lock. The mean fibre weight per centimetre was

* There is one more source of error, *viz.*, the variation of the fibre weight along the length of the fibre, which is not eliminated in this method.

first determined by the cutting method described in the Technological Reports on Standard Indian Cottons, 1928 [Turner, 1928]. The average number of fibres according to the ordinary cutting method (B) was then calculated by dividing the lint weight per seed by the product of the above mean fibre weight per centimetre and the mean fibre length in centimetres. Then using the same sliver once again, the number of fibres according to Turner's method (A) was evaluated by following the procedure described in the paper by the writer and Turner [1930]. With the values of the mean unit fibre weight obtained in this method, the average number of fibres according to the method (D) was also calculated. Unfortunately the determinations according to the method (C) could not be carried out on the same slivers as were used in the previous determinations, because the latter work had been completed much earlier than the announcement of the method of weighing whole fibres by Nazir Ahmad [1931], and the same slivers were not available when the work according to the method (C) was contemplated. Thus the results obtained by the methods (A), (B) and (D) alone are directly comparable and they are discussed in the following paragraphs.

Discussion of results.

The results obtained by the methods (A) and (B) are given in Table I.

Table I.

Position of seed in lock	Co. 2			Karunganni 546		
	No. of fibres according to the method (A)	No. of fibres according to the method (B)	Percentage of difference between methods (A) and (B) $\frac{(A)-(B)}{(A)} \times 100$	No. of fibres according to the method (A)	No. of fibres according to the method (B)	Percentage of difference between methods (A) and (B) $\frac{(A)-(B)}{(A)} \times 100$
I.	12,340	12,100	+1·95	3,300	3,180	+3·64
II.	13,130	12,830	+2·28	3,320	3,220	+3·01
III.	14,220	13,940	+1·97	3,490	3,400	+2·58
IV.	14,810	14,540	+1·82	3,770	3,740	+0·80
V.	15,300	14,870	+2·81	3,870	3,870	+0·00
VI.	15,910	15,550	+2·26	4,220	4,120	+2·37
VII.	15,370	14,900	+3·00	3,990	3,990	+2·26
VIII.	16,270	15,710	+3·44	..	..	..
IX.	15,040	14,820	+1·46	..	..	..
Mean . . .			+2·34			+2·09
Standard error of mean . . .			0·22			0·48
t. . . .			10·64			4·35
t for $P=·01$ . .			3·355			3·707.

Using the t-P criterion for estimating the significance of the differences between the values given by the two methods, it will be seen that the observed values of t are 10·64 and 4·35 for Co. 2 and Karunganni respectively, their values of t for $P=0·01$ being 3·355 and 3·707. The observed differences are therefore significant and it can be confidently stated that the values given by the method (A) are reliably greater than those given by the method (B).

This finding led the writer to consider what would be the kind of difference between the values got by Turner's method and those got by dividing the lint weight per seed by the mean unit fibre weight got in Turner's method itself. that is. between the values got by methods (A) and (D). The results for these are given in Table II.

TABLE II.

Position of seed in lock	Co. 2			Karunganni 546		
	(A)	(D)	$\frac{(A)-(D)}{(A)} \times 100$	(A)	(D)	$\frac{(A)-(D)}{(A)} \times 100$
I.	12,340	11,990	+2·84	3,300	3,210	+2·73
II.	13,130	12,840	+2·21	3,320	3,200	+3·62
III.	14,220	13,900	+2·25	3,490	3,390	+2·87
IV.	14,810	14,370	+2·97	3,770	3,660	+2·92
V.	15,300	14,920	+2·48	3,870	3,760	+2·84
VI.	15,910	15,540	+2·32	4,220	4,100	+2·84
VII.	15,370	15,110	+1·95	3,990	3,890	+2·51
VIII.	16,270	15,870	+2·46	..	..	..
IX.	15,040	14,550	+3·26	..	..	..
Mean . . .			+2·53			+2·90
Standard error of mean . . .			0·14			0·13
t. . . .			18·07			22·30
t for $P=·01$ . .			3·355			3·707

Surprisingly enough the results show the existence of the same kind of difference as was observed in the previous case but with greater consistency and uniformity.

It may now be mentioned that the figures given by Ayyar and Ayyangar [1933] were obtained by the method (A) while those derived by Barritt [1933] were by the method (D). A nearly constant difference has been found to exist between the values obtained by the two methods (A) and (D), this being due merely to the differences in the experimental methods employed. The changes in the assumptions that are responsible for this variation will be considered in a later portion of this paper.

The values obtained according to the methods (B) and (D) may now be compared. The results are recorded in Table III.

TABLE III.

Position of seed in lock	Co. 2			Karunganni 546		
	(D)	(B)	$\frac{(D)-(B)}{(D)}\times 100$	(D)	(B)	$\frac{(D)-(B)}{(D)}\times 100$
I . . .	11,990	12,100	—0·92	3,210	3,180	+0·93
II . . .	12,840	12,830	+0·08	3,200	3,220	—0·63
III . . .	13,900	13,940	—0·29	3,390	3,400	—0·30
IV . . .	14,370	14,540	—1·18	3,660	3,740	—2·18
V . . .	14,920	14,870	+0·34	3,760	3,870	—2·92
VI . . .	15,540	15,550	+0·06	4,100	4,120	—0·49
VII . . .	15,110	14,900	+1·39	3,890	3,900	—0·26
VIII . . .	15,870	15,710	+1·01	..	..	..
IX . . .	14,550	14,820	—1·86	..	..	..
Mean . . .			—0·15			—0·83
Standard error of mean.			0·35			0·49
t. . . .			0·428			1·695
t for $P=·01$.			3·355			3·707

It will be seen that the differences between the values obtained by the two methods are not consistent, some being positive in sign and some others negative. The mean values of the differences are well within the limits of experimental error.

A theoretical aspect of the foregoing differences may now be considered.

If L represents the lint weight per seed, p the percentage by weight of a particular group length, as obtained by Balls Sorter, divided by 100, and w the fibre

weight per centimetre of a particular group length l, the calculations according to the different methods may be algebraically expressed as follows :—

$$N_{(A)} = \Sigma \frac{L.\,p}{w.\,l} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (A)$$

$$N_{(B)} = \frac{L}{\bar{w}.\,\bar{l}} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (B)$$

$$N_{(D)} = \frac{L}{\frac{\Sigma p.\,w}{\Sigma p} \cdot \frac{\Sigma\, p.\,l}{\Sigma p}} \quad . \quad . \quad . \quad . \quad . \quad (D)$$

where $\bar{w}$ and $\bar{l}$ signify the average values of w and l respectively, taking into account the distribution of length as obtained by the Balls Sorter.

(D), when simplified, becomes $\frac{L}{\bar{w}'\,\bar{l}}$, where $\bar{w}'$ is the average fibre weight per centimetre taking into consideration the variation of fibre weight with the length of the fibre. When a sufficiently large number of fibres are weighed for each group length, the value of $\bar{w}'$ obtained will tend to coincide with that of $\bar{w}$. In such a case the average number of fibres per seed obtained by the method (D) will be nearly the same as that got by the method (B). This is found to be actually the case in the figures recorded in Table III. The small differences observed may be partly due to the difference between the values of $\bar{w}$ and $\bar{w}'$ and partly to the errors of the experiment.

(A) may be put in the form $\Sigma \frac{\frac{L}{w.\,l}}{p}$ which is similar to (D), except for the fact, that in (A) the actual values are taken in place of average values in (D). If the product $w.\,l$ were constant, that is, if w varied inversely as l, then (A) would be $\frac{L}{w.\,l}\,\Sigma p$ and since Σp is obviously unity, it would be $\frac{L}{w.\,l}$ or $\frac{L}{\bar{w}.\,\bar{l}}$; in which case (A) would give values nearly the same as (D). But it was found by the writer and Turner [1930], that in no case does w vary inversely as l and that the product wl decreases always with decrease in the value of l. Hence when the number of fibres is calculated for each group length and added together, as is done in the method (A), the sum would be more than the value got by using the average figures for w and l, as in (D). In fact, the situation will be similar to that represented in Fig. 4 of Iyenger and Turner's [1930] papers. (A) takes into account the distribution according to number of fibres while (D) that according to weight. It is seen that the first curve lies above the second in the smaller values of l and below it in the larger values. This is tantamount to a slight decrease in the average length, when it is

calculated according to the distribution of the number of fibres. The smaller value of l, in the denominator of the expression (A), is responsible for the higher value of the number of fibres obtained by the method (A).

One would also expect that some of these differences might be caused by a similar variation in the value of the mean fibre weight per centimetre. But on actual calculation the variation in the fibre weight has been found to be negligible and hence its effect is insignificant.

We may now consider the effect of the above considerations among the different strains of cotton. As only two strains are studied in the present work, the results recorded in the previous paper may be utilised for this purpose. These results are given in Table IV.

TABLE IV.

Part	Relation of w and l	No.	Strain of cotton	Season	(A)	(D)	$\frac{(A)-(D)}{(A)} \times 100$	Mean
I.	w increasing with decrease of l.	1	A. 19 . .	27-28	5,500	5,400	+1·8	
		2	C. A. 9 . .	27-28	7,680	7,520	+2·1	
		3	Gadag 1 . .	27-28	12,870	12,500	+2·9	
		4	Mollisoni .	27-28	5,110	4,960	+2·9	
		5	,, .	26-27	5,040	4,880	+3·2	+3·4
		6	P.-A. 4 F. .	27-28	8,670	8,390	+3·2	
		7	P.-A. 289 F. .	27-28	9,000	8,700	+3·3	
		8	C. A. 9 . .	26-27	8,950	8,570	+4·2	
		9	Co. 1 . .	27-28	14,080	13,360	+5·1	
		10	P.-A. 4 F. .	26-27	7,190	6,770	+5·8	
II.	w constant in all lengths.	1	Wagad 8 . .	26-27	10,640	10,230	+3·9	
		2	Dharwar 1 .	26-27	4,160	3,970	+4·6	
		3	H. 25 . .	26-27	3,710	3,540	+4·6	
		4	N. 14 . .	27-28	2,830	2,700	+4·6	
		5	,, . .	26-27	2,500	2,380	+4·8	
		6	Surat 1027 A. L. F.	26-27	5,840	5,560	+4·8	+5·0
		7	Karunganni C. 7	27-28	5,230	4,980	+4·8	
		8	Gadag 1 . .	26-27	13,170	12,510	+5·0	
		9	Dharwar 1 .	27-28	4,220	3,950	+6·4	
		10	Karunganni C. 7	26-27	5,000	4,670	+6·6	
III.	w decreasing with decrease of l.	1	Wagad 4 . .	27-28	7,170	6,600	+7·9	
		2	Do. . .	26-27	9,030	8,300	+8·1	+8·0
IV.	w constant but distribution of l very heterogeneous.	1	P.-A. 285 F. .	26-27	8,690	7,800	+10·2	
		2	P.-A. 289 F. .	26-27	8,540	7,500	+12·2	+11·2

It is clear from the foregoing considerations that greater differences are to be expected between (A) and (D), when the shift between the two fibre-length distribution curves increases. It is also clear from the arguments put forward in the previous paper [Iyenger and Turner, 1930], that the shift between the curves should increase from Part I to Part III of Table IV. It therefore follows that the difference between (A) and (D) also should increase in that order, which is indeed found to be the case.

If the mode of variation of w with respect to l were the only cause, we have to expect every value of Part I to be greater than every value of Part II, which in turn should be greater than every value of Part III. The actual figures, however, do not follow such an order. The reason for this is the fact, that the variation of w is not the only cause, but that the distribution of length also adds its share towards this difference. This can be clearly seen in the values found in Part IV, where P.-A. 289 F. and P.-A. 285 F., on account of the very heterogeneous distribution of their fibre length (Fig. 3 of the previous paper), have recorded high differences of 10 to 12 per cent.

It may be stated here that in the method (C), where whole fibres are weighed, the error in the calculation of the mean fibre length does not arise and the error due to the variation of the weight along the length of the fibre is also eliminated. Hence from *a priori* considerations this method appears to be very sound, provided a sufficiently large numbers of fibres are weighed, so as to make the fibres weighed really representative of the sample. This method has the added advantage of being very simple also. Unfortunately comparative values are not available to substantiate this expectation.

Conclusion.

The average number of fibres per seed according to the ordinary cutting method (B) is a little smaller than that obtained by the method suggested by Turner (A). Where considerable accuracy is not needed, the ordinary cutting method may be employed with a positive correction of about 2 to 3 per cent.

The values obtained according to the method (D) also, are less than those got by (A) by about the same amount. This difference has been shown to be due to the fact that (A) takes into consideration the distribution of the fibre-length according to the number of fibres, while (D) that according to weight and that there exists a slight variation between the two distributions.

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THE EFFECT OF HYDROGEN ION CONCENTRATION ON THE GROWTH OF *HELMINTHOSPORIUM NODULOSUM* B. ET C. AND *H. LEUCOSTYLUM* DRECH.

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(With one text-figure)

Introduction.

Several investigators have studied the influence of the reaction of nutritive media on the growth of fungi. Kirby [1922, 1925] found that *Ophiobolus graminis* Sacc. grows best on a highly alkaline medium, whereas Davis [1925] has shown that a neutral or slightly acid medium is most favourable for the growth of *Ophiobolus graminis*. Experiments carried out by McKinney [1925] indicate that the growth of *Helminthosporium sativum* P. K. and B. is favoured by a neutral to slightly acid medium. Dosdall [1923] found that in phosphoric acid-potassium hydroxide solutions, the spores of *Helminthosporium sativum* germinated through a wide range of hydrogen-ion concentrations. A double optimum occurred both maxima falling on the alkaline side of neutrality at pH 8·2 and pH 9·2. In Czapek's solution *minus* the sugar, the maximum germination occurred at pH 6 and pH 8. In general she concludes that the spores germinate better in alkaline solutions than in acid solutions and tolerate high degree of alkalinity. On the contrary Webb [1919-1921] has shown that germination is strikingly supported by a relatively high hydrogen-ion concentration, and hence hydroxyl-ions are more toxic than hydrogen ions. Leach [1923] also found that *Colletotrichum lindemuthianum* (Sacc. & Mag.) Br. & Cav. grew best in an alkaline medium and that when growth occurred in an acid medium it was turned alkaline. Webb and Fellows [1926] showed that the influence of the hydrogen-ion concentration on the vegetative growth of *Ophiobolus graminis* is variable, and depends to a large extent on the chemical composition and the physical nature of the medium. Dennis [1933] found that optimum growth for *Helminthosporium avenae* Eidano lies about pH 6, that is, on the acid side and that satisfactory growth may be expected between pH 5 and

pH 6·75. Singh [1933] found in the case of *Cercospora dolichi* E. and E. the range of pH from 3·3-8·1 and he obtained two maxima, one at pH 4·6 and the other at pH 7·1 and concluded that this fungus required for its best growth an acid or a slightly alkaline medium. In the present study the effect of hydrogen-ion concentration on the growth of *Helminthosporium nodulosum* B. et C. and *H. leucostylum* Drech. isolated from *Eleusine coracana* was studied.

SPORE GERMINATION.

Spores of *H. nodulosum* germinated fairly well in tap water, the pH of which in Pusa is 8·0-8·1. In order to determine more accurately the tolerance of active acidity and alkalinity by the spores, a study of germination in solutions of varying hydrogen-ion concentrations was taken up. A series of solution of different pH values was made up by adding various amounts of $N/5$ hydrochloric acid and potassium hydroxide to two per cent. bacto-peptone solution following approximately the curve given for this medium by Karrer and Webb [1920]. Spore suspensions were made in solutions of known pH values and a drop mounted on a cover slip and inverted over Van Tieghan rings containing a few drops of solution of the same pH value as of the hanging drop. Four slides were used for each test and incubated for 2½ hours at 30°C. The percentage of germination and average of 200 measurements for length of germ tubes were taken. The experiment was repeated thrice. The results of the experiment are given in Table I.

TABLE I.

Spore germination of H. nodulosum *in bacto-peptone solutions of various hydrogen-ion concentrations after 2½ hours at 30°C.*

pH	Germination percentage	Average length of germ tubes in microns.
1·8	0·0	0·0
2·2	0·0	0·0
3·8	71·4	114·0
4·0	93·2	152·6
4·5	90·0	152·4
5·6	88·2	182·4
6·5	95·5	220·0
6·9	95·8	212·0
7·9	93·7	152·0
8·3	88·0	136·8
8·5	85·6	124·4
9·1	76·2	111·6
9·9	66·0	66·0
10·0	17·0	28·0

After an incubation of twenty-four hours at 30°C. no germination was obtained up to pH 2·2. Germination was fair at pH 3·8, and high from pH 4·0-pH 7·9 above which it gradually declined till at pH 10·0 it was very little.

It was noted that between pH 3·8 and 4·5 (*i.e.*, acid range) the germ tubes were very much branched and consequently the main germ tube was not so well developed. This was not noted in neutral or alkaline cultures. In the latter the germ tubes were straight and long. It is therefore difficult to fix an exact optimum for the best germination but it lies within a range of pH 4·0-7·9.

It is not possible to state if the relation of germination of the spores of *H. nodulosum* to hydrogen-ion concentration is similar or different in soil solutions. All that can be inferred from the present studies is that the spores can germinate over a wide range of hydrogen-ion concentrations.

Mycelial growth.

The reaction of the substratum is recognised as one of the most important environmental factors influencing the physiology of the living organisms. It has been pointed out that the "active" acidity and alkalinity influence the reaction of the substratum, and Fuller's scale where total acidity and alkalinity is taken into consideration is not a reliable index of the true pH value of the medium.

A study of the growth of *H. nodulosum* and *H. leucostylum* in relation to hydrogen-ion concentration was made. Richards' solution was the medium chosen for the experimental work, its composition being the same as given by Karrer and Webb [1920], and the culture solutions were made according to the method described by them.

The fungus was grown in this medium in 250-c.c. flasks which had been thoroughly cleaned. As the figures in the titration table given by Karrer and Webb [1920] differed from those obtained in this work, a new titration table was made with Merck's chemicals, and followed in these experiments. The pH value determinations were made colorimetrically with the "Hellige Comparator" with standard discs. All the inoculated flasks with controls, each of which contained 50 c.c. of the medium of different pH values, were incubated at 30°C. in triplicate for a period of twenty-two days and the dry weight of the fungus, pH of the control and that of filtrate were obtained. The data obtained are given in Table II and Fig. 1.

TABLE II.

Growth of H. nodulosum *and the changes in reaction induced by growth in modified Richards' solution at different initial pH values at 30°C. after twenty-two days.*

Culture number	Hydrogen-ion concentration			Average dry weight in grms.	Growth character
	Initial	Final after 22 days			
		Control	Inoculated		
1	2·1	2·9	2·5	0·000	No growth.
2	2·9	2·9	3·0	0·029	Growth poor and submerged, colony as many small balls of pale or yellow colour.
3	4·6	6·3	2·9	0·082	Growth both floating and submerged. Floating colonies of flesh colour, white gelatinous submerged growth.
4	5·5	6·5	3·0	0·098	Growth very much like above. Submerged mycelium of light gray colour.
5	6·3	6·5	3·2	0·089	Few broad white floating colonies and submerged white and gelatinous growth.
6	6·5	6·7	3·2	0·127	Few broad floating colonies as in above but of darker colour. Floating colonies of plumbago blue and submerged growth of smoke gray.
7	7·1	6·9	3·2	0 185	Growth as in above, submerged colony of ivory black colour.
8	7·5	6·9	3·4	0·143	Many small and thick floating colonies of ivory black colour.
9	8·2	7·3	7·3	0·000	Growth in traces.
10	8·5	7·3	7·3	0·000	No growth.
11	9·3	8·1	8·4	0·000	No growth.

From the table and Fig. 1 it will be noticed that the dry weight gradually increases with the decrease of acidity, and the maximum growth was obtained at pH 7·1. It then decreased rapidly to a minimum at pH 7·5.

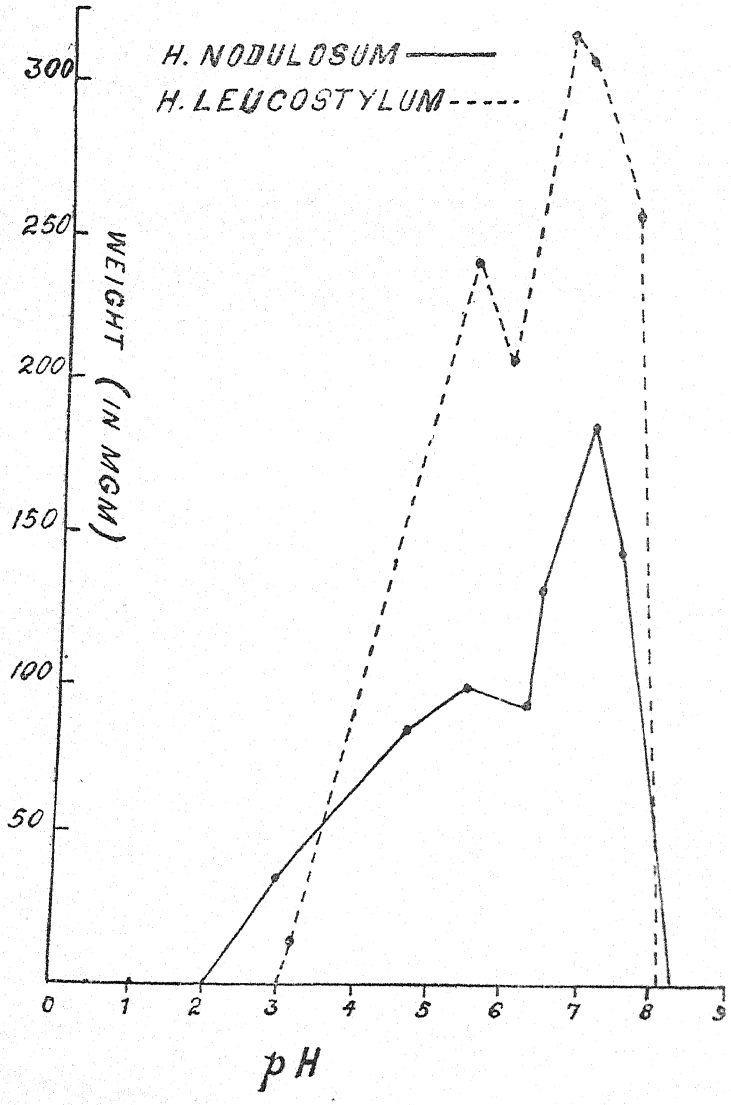


Fig. 1.—The growth of *H. nodulosum* and *H. leucostylum* in Richards' solution at different pH values at 30°C. after twenty-two days.

The growth of the fungus changed the pH value of the medium. There was only a slight change in the most acid solution while the alkaline solutions at the extreme end showed most marked change. It will be observed that even in the uninoculated culture flasks there is a shift in the pH in most cases towards neutrality. An experiment, which was carried out later on in which the shift in pH value of

the solution was tested after every week, showed that the initial pH was retained for twelve days and slight changes gradually began after this interval. The inoculum in culture flasks Nos. 1, 10, and 11 was killed on account of the toxicity of the acid and alkaline-ions. Some growth ought to have taken place in these flasks when the pH showed a shift towards neutrality where growth was possible. Culture flask No. 9 showed some growth after long incubation. Apparently the concentration of the ions was not toxic enough to kill but only to inhibit growth which took place after the pH had shifted to a higher value.

The colour of colonies differed in various ranges of pH and also on the position of the colonies. But the tendency was to form small bead like colonies of the pale flesh pink colour in acid media, gradually changing to thin silvery white in neutral, and finally to thick flat colonies of darker colour in alkaline media.

A similar procedure was adopted to find the relation of hydrogen-ion concentration to growth of *H. leucostylum* in Richards' solution as done in the previous experiment. The result is given in Table III.

TABLE III.

The growth of H. leucostylum *and the change in reaction induced by growth in modified Richards' solution at different intial pH values at 30°C. after twenty-two days.*

Culture number	Hydrogen-ion concentration			Average dry weight in grms.	Growth characters
	Initial	Final after 22 days			
		Control	Inoculated		
1	3·0	3·0	3·0	0·000	No growth.
2	4·2	5·6	4·3	0·013	Small floating colonies of flesh colour.
3	5·5	6·0	3·4	0·238	Broad, flat and lobed colonies of light rose.
4	6·0	6·2	3·9	0·205	Growth as in above.
5	6·7	6·9	4·5	0·314	Many flat lobed colonies of pale lilac colour.
6	7·0	6·8	4·6	0·306	Few big colonies of rosy scarlet colour.
7	7·7	6·8	4·6	0·252	Few compact broad colonies of pale rosy pink.
8	8·1	6·8	6·8	Trace	Growth in traces.
9	8·5	6·8	6·8	0·000	No growth.

The results of the experiment showed that the fungus grew within a range of hydrogen-ion concentration from 4·2-8·0, maximum growth showing at pH 6·7 and

good growth between pH 5·5-7·7. The growth of the fungus resulted in the shift of the pH value of the culture solution towards acid side. On the whole the change of pH value in cultures of *H. leucostylum* was not so marked as in that of *H. nodulosum*. The change in colour at varying pH values was also less marked than in *H. nodulosum*. The colour was pink in all the cultures and became intensified with increase in pH values reaching maximum depth at pH 7·0 and again becoming lighter in alkaline cultures.

Conclusion and Summary.

From the study of the relationship of hydrogen-ion concentration it was observed that the spores of *H. nodulosum* tolerate a wide range of pH, that is, from 3·8-10, the optimum lying between 6·5-6·9 both for the percentage of germination and for length of germ tube. The dry weight of *H. nodulosum* on Richards' solution of various hydrogen-ion concentration showed that the optimum is at pH 7·1 and that for *H. leucostylum* at 6·7. Further it was noticed that the pH value of the medium changes with the growth. The pH of control flask also changed. Further it was observed that pH has no effect on the colour of the colony as well. Thus it is seen that too high hydrogen-ion and too low hydroxyl-ion concentrations are harmful to the best growth of the fungus.

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NOTE

SOIL PROFILES IN THE HIMALAYAS.

A paper in the *Indian Forester* for June 1934 entitled "An Investigation of some Bajrundi Forest Soils with reference to Regeneration of Spruce Fir, *Picea morinda*" by E. Mackenzie Taylor, M. L. Mehta and R. C. Hoon, is of considerable interest to agricultural research workers, not only because of the connection established between soil characters and regeneration but because very few profiles of Himalayan hill soils have been examined in detail.

TECHNICAL COMMUNICATION NO. 10 OF THE IMPERIAL BUREAU OF SOIL SCIENCE.

It has been brought to our notice that a number of agricultural officers and others interested in the conduct of field experiments are not familiar with Technical Communication No. 10 of the Imperial Bureau of Soil Science, "The Arrangement of Field Experiments and the Statistical Reduction of the Results" by Dr. R. A. Fisher, Sc.D., F.R.S., and Dr. J. Wishart, D.Sc., which was published in 1930. Many copies were supplied free when this bulletin first appeared and the Imperial Council of Agricultural Research distribution list shows that it should be available in every Agricultural College library in India. A further supply has now been arranged and additional copies can be purchased at cost price, *viz.*, 12 annas. Applications should be addressed to the Secretary, Imperial Council of Agricultural Research, Publication Branch, Imperial Record Department Building, New Delhi and accompanied by a remittance.

Technical Communication No. 10 describes in the simplest possible language the principles underlying the Field experimental technique elaborated at Rothamsted and gives with appropriate illustrations from actual experiments the full arithmetical working involved in the statistical reduction of the data. The use of Randomized Blocks and the Latin Square Method of layout is explained and the arithmetical working up of the results including the analysis of variance. No mathematical knowledge on the part of the reader is assumed. This short bulletin should be on every agricultural officer's bookshelf.

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* '*Agriculture and Livestock in India*,' '*The Indian Journal of Agricultural Science*' and '*The Indian Journal of Veterinary Science and Animal Husbandry*' replaced the following publications of the Imperial Department of Agriculture in India with effect from January 1931—

1. The Agricultural Journal of India.
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ORIGINAL ARTICLES

AGRICULTURAL METEOROLOGY: PRELIMINARY STUDIES ON SOIL-MOISTURE IN RELATION TO MOISTURE IN THE SURFACE LAYERS OF THE ATMOSPHERE DURING THE CLEAR SEASON AT POONA.

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(Received for publication on 30th May 1934)

(With four text-figures).

I. INTRODUCTION.

It is well known to soil investigators that a dried sample of soil absorbs moisture from the atmosphere until the hygroscopic equilibrium for the prevailing temperature of the soil is reached. Various investigations [Keen, 1931] have been made in the laboratory in order to determine the hygroscopic properties of different soils. It is, however, not usually recognised that these properties may have a meteorological significance in so far as the exchange of water vapour between the soil and the air layers near it is concerned. In order to investigate the intensity and variation of different meteorological elements within the first two metres above ground, the Agricultural Meteorology Branch at Poona has been recording daily observations at its observatory which is situated in the farm of the local Agricultural College. These detailed observations reveal various interesting features which are of ecological importance [Ramdas, Kalamkar and Gadre, 1934]. In the present paper attention is confined to moisture relations between the soil and air as deduced from observations of dry and wet bulb temperatures taken with an Assmann Psychrometer at short height intervals above the ground surface and observations of soil moisture. It is observed that the vapour pressure decreases with height during the day and increases with height during the night. The explanation of the

increase of vapour pressure with height above ground during night was somewhat puzzling at first. To account for the phenomenon the possibility of drier air at higher levels cooling more than the air layers near the ground by radiation just after sunset and settling down was investigated by taking detailed measurements of the fall of temperature and the distribution of vapour pressure at very quick intervals. No evidence for such settling down of drier air from above could be established. As soon as measurements of evaporation and absorption at the ground surface began to be made it was felt that the real explanation might be sought for in that direction. In the following sections the distribution of soil moisture and temperature above and below the ground will be first mentioned and then the main subject of the paper taken up for discussion.

II. Soil moisture.

After the occasional thunderstorms in April and May and later during the monsoon season (June-September), the soil in the Bombay Deccan is usually wet the actual moisture content at various depths below the surface depending on the distribution and intensity of the rainfall. Later, from November onwards the skies are generally clear or lightly clouded, and, owing to solar heating, the top layer of the soil becomes rapidly desiccated. During winter and spring (December to April) until the thunderstorm season begins the surface of the ground is subjected to intense heating during the day. One may expect that during this period the top-most layer of the soil will be so desiccated that it retains only the minimum of water determined by its hygroscopic properties. Table I gives estimates of water content of the soil at different depths during representative months in 1933-34. The values are expressed as percentage of the dry weight of the soil samples which were dried in the usual manner by keeping in a steam-heated oven until the dry weight ceases to decrease any further. Samples were taken once a week from the bare plot in which the observatory is situated.

Table I.

Depth (in.)	January 1933	July 1933	September 1933	November 1933	January 1934	March 1934
0	5	21	27	11	6	6
2	19	29	36	12	15	8
4	23	30	32	21	19	11
6	25	29	33	25	22	14
12	27	28	32	26	24	26

It will be seen that whereas during the monsoon the water content goes up to 36 per cent. and does not vary much with the depth, during the winter and spring

the percentage of water at the top of the soil is as low as about 6 per cent. but increases rapidly with depth. It is significant that even after two or three months of desiccation after the rainy season is over, the moisture content of the soil at a depth of one ft. has decreased only slightly and is still as high as about 25 per cent.

This fact is of great importance in dry farming districts where the conservation of moisture in the soil is the main consideration and where the soil surface is allowed to dry up as rapidly as possible in order to form a protecting layer [Widtsoe, 1920].

III. Temperature.

During the monsoon period with its steady and strong air movements, overcast skies and frequent drizzle or rain, the air as well as the soil are more or less in a saturated state so far as moisture is concerned and there is comparatively little variation of temperature which is fairly low. When the clear season begins the unbroken sunshine promotes evaporation from the soil and the diurnal variation of temperature begins to increase rapidly as the season advances. Table II shows the annual march of mean daily air temperature and the diurnal range of temperature * at a level of 4 ft. above ground.

Table II.

Month	Mean daily temperature °C.	Diurnal range °C.
January	21·2	17·8
February	23·0	19·2
March	26·6	19·1
April	29·4	17·9
May	29·9	15·4
June	27·3	9·4
July	24·9	6·5
August	24·2	6·7
September	24·7	8·9
October	25·4	12·5
November	22·8	15·2
December	20·8	17·1

* Based on 43 years' data recorded at Yervada, Poona.

Whereas north India comes under the influence of the winter depressions and south-east India is affected by the N.-E. monsoon during the early part of winter, the Bombay-Deccan and its adjoining tracts enjoy comparatively clear and calm weather. The winter and early spring seasons of the Deccan approximate therefore to a "mathematical climate" or a climate of the "insolation" type modified only slightly by air movements. Very high soil temperatures are experienced owing to the uninterrupted sunshine which is almost completely absorbed by the black cotton soil surface. Fig. 1 shows the hourly variation of the soil surface temperature on 28th March 1934 as recorded by a soil thermograph. At the maximum epoch the temperature is as high as 74°C. The temperature falls rapidly after sunset and is as low as 16°C. at the minimum epoch. The diurnal variation at the surface is of the order of 58°C. which corresponds to nearly 100°F.

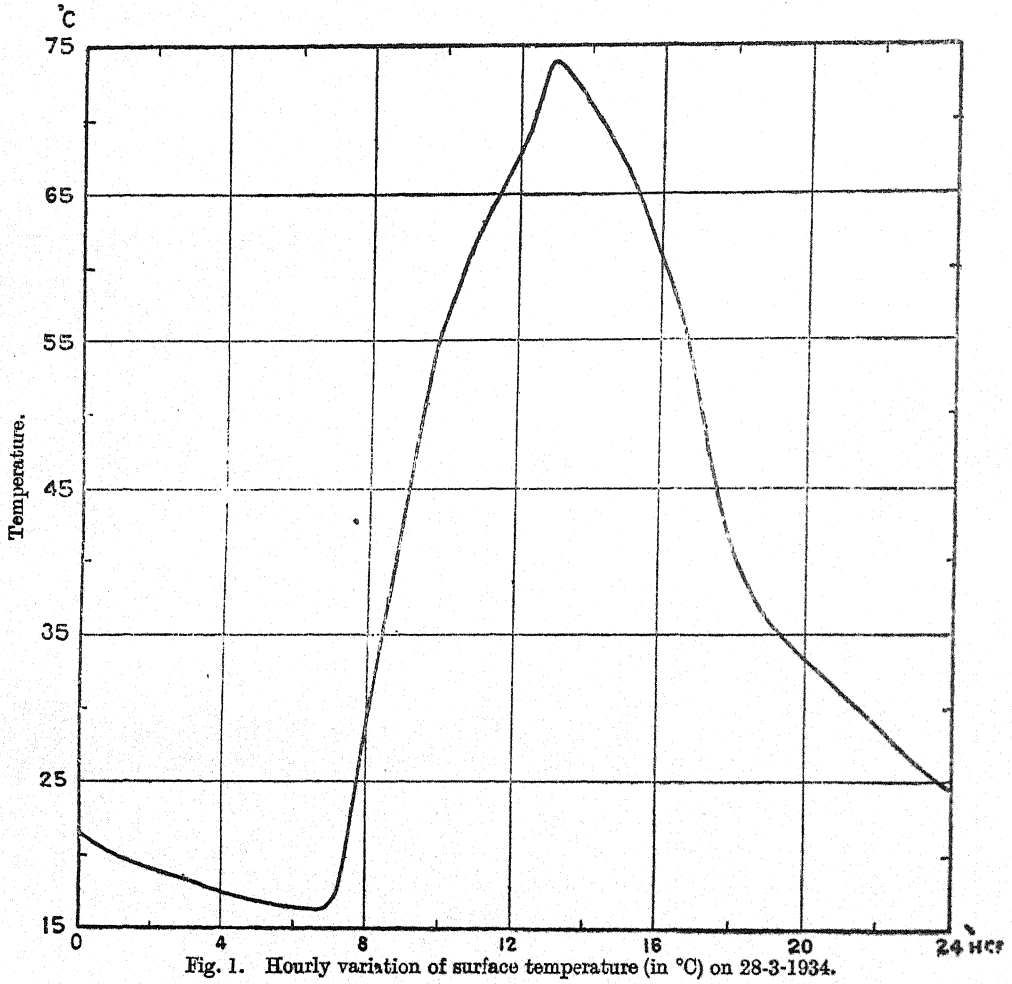


Fig. 1. Hourly variation of surface temperature (in °C) on 28-3-1934.

This shows the very intense heating to which the soil surface is subjected during the day hours. The diurnal variation, of course, decreases rapidly with depth so that the desiccation during the day is confined mainly to the surface layer of the soil.

The hourly march of air and soil temperatures at various heights and depths respectively is also brought out by Fig. 2 where isopleths of the 5-day averages of hourly temperatures taken from 4th to 8th January 1933 have been plotted. The high positive lapse rates at mid-day and the nocturnal inversion are clearly brought out by this diagram. These data will be discussed in greater detail elsewhere.

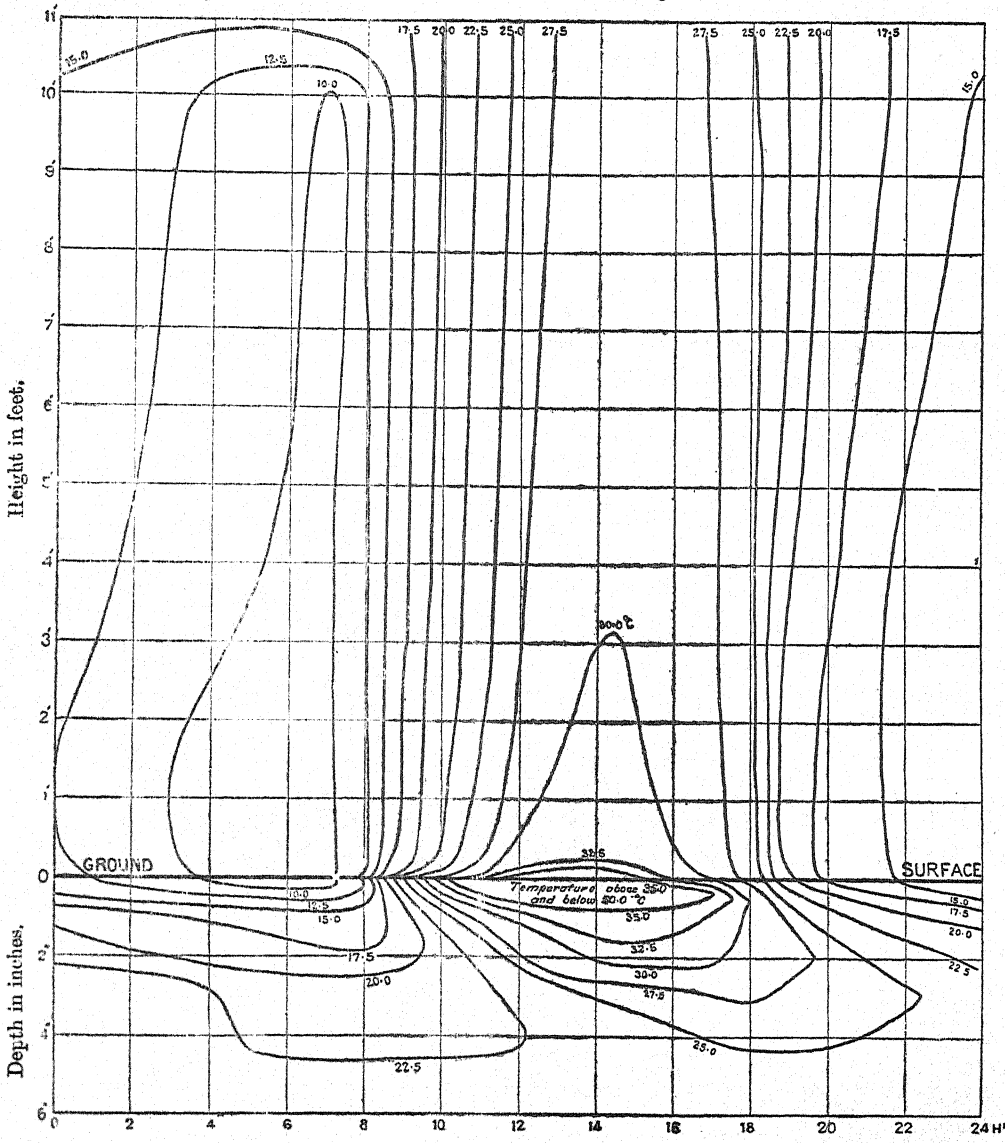


Fig. 2. Mean hourly variation of temperature (in °C) above and below ground during the period 4-1-33 to 8-1-33.

IV. Vapour pressure and humidity.

We may now consider the variation of vapour pressure and humidity with height at the epochs of maximum and minimum temperatures. Table III gives the mean monthly values of vapour pressure and percentage humidity at different levels during the period January to April 1933 at the maximum temperature epoch and Tables IV and V give similar data for the same period and for the period November 1933 to April 1934, respectively, at the minimum temperature epoch. Table III shows that vapour pressure is highest near the ground during the afternoon and that it decreases with height; the percentage humidity, however, is a minimum near the ground and increases with height. This shows that the evaporation from the soil is not sufficiently large to keep the hot air near the ground saturated to the same extent as the cooler layers higher up.

Table III.

Mean vapour pressure in mm. of mercury and percentage humidity at different levels—Maximum temperature epoch.

Height above ground	January 1933		February 1933		March 1933		April 1933	
	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.
0·3 in. . . .	8·5	18	6·8	13	5·7	8	8·3	11
1·0 in. . . .	8·0	19	6·5	14	5·6	9	7·9	13
3·0 in. . . .	7·8	21	6·4	15	5·5	10	8·0	14
6·0 in. . . .	7·8	22	6·2	15	5·5	11	8·0	15
1 ft.	7·8	23	6·2	16	5·5	11	8·0	15
2 ft.	7·7	23	6·1	17	5·5	12	7·8	16
3 ft.	7·6	24	6·1	17	5·5	12	7·8	17
4 ft.	7·6	25	6·1	17	5·2	12	7·7	17
6 ft.	7·5	25	5·9	17	5·2	12	7·6	17
8 ft.	..	..	5·9	18	5·2	13	7·6	17
10 ft.	..	..	5·8	18	5·1	13	7·5	17

TABLE IV.

Mean vapour pressure in mm. of mercury and percentage humidity at different levels—Minimum temperature epoch.

Height above ground	January 1933		February 1933		March 1933		April 1933	
	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.
0·3 in. . . .	5·8	64	6·5	62	6·1	48	8·9	49
1·0 in. . . .	5·9	66	6·2	60	6·1	47	9·3	49
3·0 in. . . .	5·9	66	6·3	62	6·1	48	9·3	50
6·0 in. . . .	6·0	69	6·3	64	6·3	50	9·4	50
1 ft.	6·3	72	6·6	67	6·3	50	9·4	51
2 ft.	6·6	74	7·0	69	6·5	50	9·5	51
3 ft.	6·9	75	7·3	70	6·9	52	9·7	52
4 ft.	7·1	76	7·4	70	7·1	53	9·8	52
6 ft.	7·4	77	7·8	71	7·3	54	10·0	52
8 ft.	..	..	8·1	71	7·6	55	10·2	53
10 ft.	..	..	8·3	72	7·7	55	10·2	53

TABLE V.

Mean vapour pressure in mm. of mercury and percentage humidity at different levels—Minimum temperature epoch.

Height above ground	November 1933		December 1933		January 1934		February 1934		March 1934		April 1934	
	Vapour pressure	Humidity per cent.	Vapour pressure.	Humidity per cent.	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.	Vapour pressure	Humidity per cent.
0·3 in.	10·4	80	7·9	80	6·7	72	4·4	49	5·7	45	10·2	58
1·0 in.	10·3	81	7·6	80	6·7	75	4·3	50	5·7	46	10·3	59
3·0 in.	10·3	81	7·7	80	6·7	75	4·5	53	5·7	47	10·3	59
6·0 in.	10·3	82	7·9	82	6·8	76	4·5	53	5·9	48	10·4	60
1 ft.	10·5	82	8·0	83	6·9	78	4·8	54	6·0	49	10·6	60
2 ft.	10·7	84	8·2	84	7·1	79	5·0	56	6·3	50	10·7	60
3 ft.	11·0	85	8·6	86	7·4	80	5·4	58	6·7	52	10·9	61
4 ft.	11·3	86	8·9	87	7·7	80	5·6	59	7·0	53	11·1	61
6 ft.	11·5	86	9·1	87	7·9	79	6·1	60	7·4	55	11·4	62
8 ft.	11·7	87	9·3	87	8·0	77	6·3	60	7·7	56	11·6	63
10 ft.	11·9	86	9·2	87	8·1	77	6·5	60	7·9	55	11·7	63

Table IV shows that in the morning the vapour pressure is a minimum near the ground and increases with height. The percentage humidity also increases with height. There is less water vapour near the ground than at the higher levels. Table V confirms the results given in Table IV.

It may be mentioned also that the regularities seen in Tables III, IV and V are of daily occurrence, so that their significance is beyond doubt.

Hourly observations of vapour pressure and humidity obtained from Assmann Psychrometer readings taken at various levels during the period 4th to 8th January, 1933, fully support the above conclusions. Figs. 3 and 4 represent isopleths of the five-day means of the vapour pressure in mm. of mercury and the percentage humidity during the above period for different hours of the day. The figures show unmistakably the gradual transition from the conditions at the maximum epoch to those at the minimum epoch, *i.e.*, the change from decrease of vapour pressure to the increase of vapour pressure with height. It will be noticed that the maximum epoch conditions begin developing by about 9 A.M. when, owing to evaporation from the soil, there is a general increase of vapour pressure at all levels. This general increase at all heights continues only so long as the winds are weak; when diurnal winds set in by mid-day and turbulence or mixing with the dry air above becomes vigorous, the increase of vapour pressure is checked but the largest value of vapour pressure continues to be near the ground. Towards sunset the vapour pressure begins decreasing near the soil and increasing at the higher levels.* The increase of vapour pressure at higher levels may be expected because towards evening and later at night the turbulence at the higher levels rapidly decreases in intensity and the mixing with drier air above also diminishes proportionately. During the transition periods, *i.e.*, just after sunrise as well as just after sunset the vapour pressure is more or less similar at all levels. Similarly the percentage humidity (Fig. 4) decreases rapidly to a minimum value of 17 per cent. near the soil at 14 hours. During the day also the percentage saturation increases with height as the temperatures near the soil (Fig. 2) are so high that in spite of evaporation from the soil the moisture present saturates the air near the ground to a less extent than at higher levels. For a few hours after sunrise and sunset respectively the humidity is more or less constant with height, but at other times the general tendency is for it to increase with height. During the night the saturation is still low near the soil. It

* Towards the end of February the Westerly sea breeze begins to arrive at Poona about sunset. This causes an increase of vapour pressure at all levels, but the absorption of water vapour by the soil and the decrease of vapour pressure with height continue to be as usual.

may be remarked here that during the early part of winter 1931, observations of dry and wet bulb temperatures had been taken regularly over a hard asphalted tennis court [Ramdas and Atmanathan, 1932], which may be expected to be comparatively impervious to the movement of water vapour unlike the ordinary soil. Table VI gives 15 days' (24th November 1931 to 11th December 1931) averages of vapour pressure and humidity at various levels. The values show clearly that over the hard surface of the court there is practically no variation of vapour pressure with height during calm weather. The fact that the soil operates in controlling the distribution of moisture in the air layers near the ground is further confirmed by the results discussed in the next section.

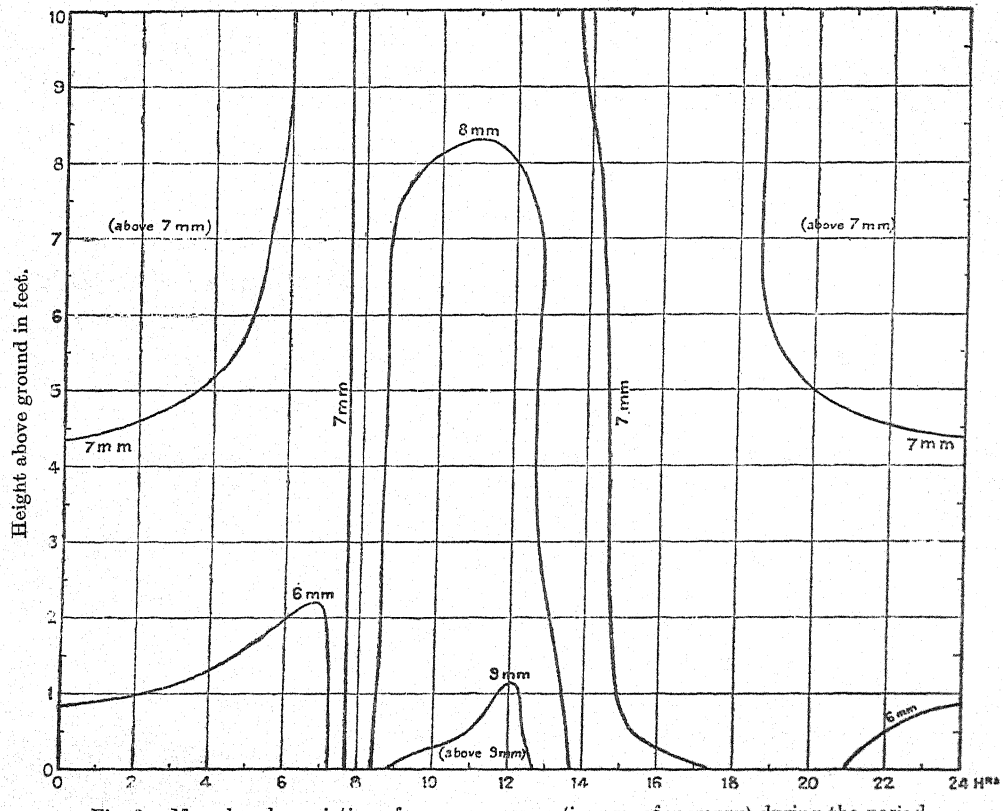


Fig. 3. Mean hourly variation of vapour pressure (in mm. of mercury) during the period 4-1-33 to 8-1-33.

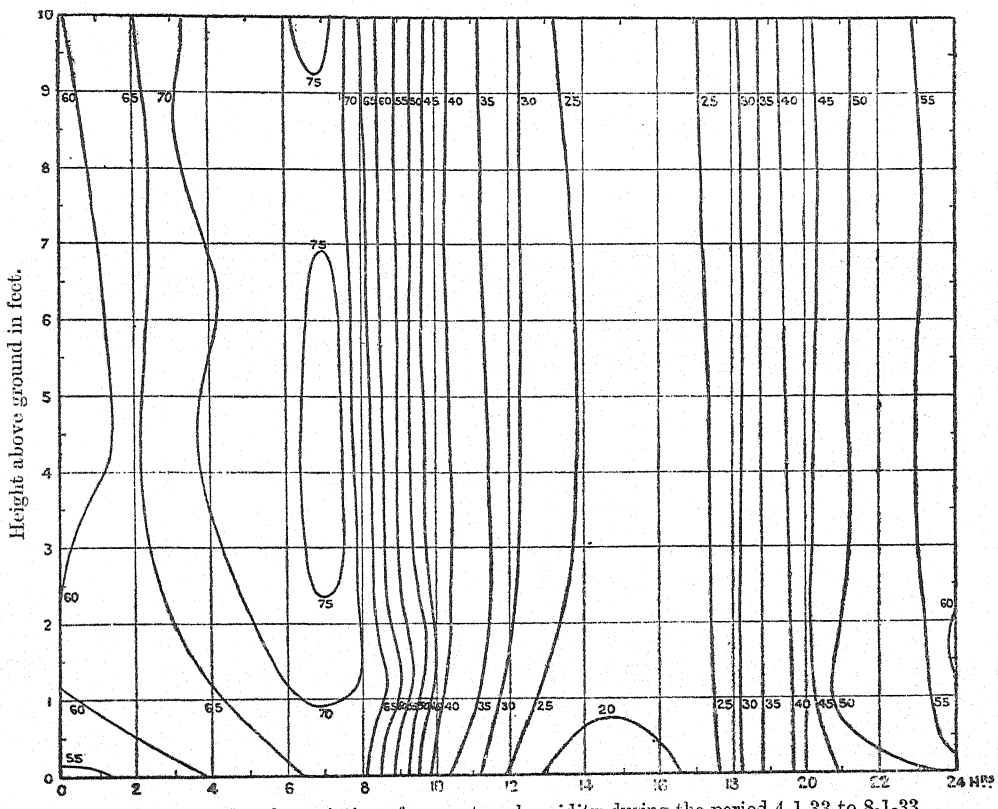


Fig. 4. Mean hourly variation of percentage humidity during the period 4-1-33 to 8-1-33.

TABLE VI.

Mean vapour pressure in mm. of mercury and humidity over tennis court (average of 15 days from 24th November, 1931 to 11th December, 1931).

Height	0·3 in.	10 in.	2 ft. 6 in.	5 ft.	7 ft. 4 in.	12 ft.
Vapour pressure	9·3	9·1	9·2	9·2	9·2	9·2
Humidity per cent.	80	87	89	88	88	88

V. Evaporation and absorption of moisture from the soil.

Direct evidence for the influence of soil as a controlling factor in the distribution of moisture in the atmosphere was also sought by experimenting with samples of known weight exposed (1) at the top of the open terrace of the Meteorological Office (height above ground 45 ft.) and (2) at the ground level in an exposed place. In the case of (1) the small vessel containing the sample was embedded in a large vessel containing sufficient depth of soil in order to prevent side effects. The surface of soil in the sample was kept level with that of the soil in the outer vessel. In the case of (2) similar samples were kept embedded in the soil at the ground surface. The area of cross-section of the vessel in which the soil was exposed was about 25 sq. cms. and the weight of the soil content was about 15 grms. in each case. The samples were carefully weighed at sunset and sunrise daily. The successive weighings gave therefore the weight of water lost by evaporation during the day and that gained by absorption during the night. Table VII shows the results obtained in the two series of experiments.

Table VII.

Date	Sample on terrace				Sample at ground level			
	Absorption		Evaporation		Absorption		Evaporation	
	Actual in grms.	Percentage	Actual in grms.	Percentage	Actual in grms.	Percentage	Actual in grms.	Percentage
February 1934—								
5	0·510	3·3	0·864	5·6	..	..	..	..
6	0·618	4·0	0·701	4·6	..	..	..	..
7	0·725	4·7	0·760	5·0	..	..	..	..
8	0·784	5·1	0·854	5·6	..	..	..	..
9	0·825	5·4	0·844	5·5	..	..	..	..
16	1·444	9·4	1·319	8·6	..	..	..	..
17	1·014	6·6	1·160	7·5	..	..	..	..
18	1·042	6·8	1·142	7·4	..	..	..	..
19	0·905	5·9	0·890	5·8	..	..	..	..
20	0·848	5·5	0·823	5·4	..	..	..	..
21	0·856	5·6	0·821	5·3	..	..	..	..
22	0·813	5·3	0·767	5·0	..	..	..	..
23	0·579	3·8	0·657	4·3	..	..	..	..
24	0·725	4·7	0·651	4·3	..	..	..	..
25	0·774	5·0	0·721	4·7	..	..	..	..
26	0·810	5·3	0·995	6·5	..	..	..	..
27	0·967	6·3	0·835	5·4	..	..	..	..
28	0·612	4·0	0·648	4·2	..	..	..	..

TABLE VII—*contd.*

Date	Sample on terrace				Sample of ground level			
	Absorption		Evaporation		Absorption		Evaporation	
	Actual in grms.	Per-centage	Actual in grms.	Per-centage	Actual in grms.	Per-centage	Actual in grms.	Per-centage
March 1934—								
1	0·606	3·9	0·620	4·0	..	..	..	..
2	0·835	5·4	0·958	6·4	..	..	..	..
3	0·871	5·7	0·774	5·0	..	..	..	..
4	0·732	4·8	0·553	3·6	..	..	..	..
5	0·685	4·5	0·770	5·0	..	..	..	..
6	0·530	3·5	0·607	3·9	..	..	..	..
7	0·763	5·0	0·818	5·3	0·720	4·7	0·892	5·9
8	0·787	5·1	0·912	5·9	0·572	3·8	0·611	4·0
9	0·802	5·2	0·885	5·8	0·651	4·3	0·630	4·1
10	0·843	5·5	0·848	5·6	0·685	4·5	0·764	5·2
11	0·780	5·1	0·702	4·6	0·684	4·5	0·645	4·2
12	0·974	6·3	0·812	5·3	0·655	4·3	0·557	3·7
14	0·808	5·3	1·092	7·1	0·683	4·5	0·938	6·2
15	0·534	3·5	0·457	3·0	0·442	3·0	0·360	2·3
20	0·964	6·3	1·004	6·5	0·825	5·4	0·878	5·8
21	0·812	5·3	0·756	4·9	0·812	5·3	0·976	6·4
22	0·792	5·1	0·894	5·8	0·654	4·3	0·647	4·3
23	0·850	5·6	0·477	3·1	0·660	4·3	0·500	3·2
24	0·688	4·5	1·008	6·6	0·557	3·7	0·809	5·3
25	0·682	4·5	0·652	4·3	0·552	3·6	0·540	3·5
26	0·565	3·7	0·562	3·7	0·475	3·1	0·482	3·2
28	0·774	5·0	0·894	5·8	0·615	4·5	0·730	4·8
30	0·550	3·6	0·745	4·9	0·629	4·1	0·752	4·9
31	1·025	6·7	0·568	3·7	0·880	5·8	0·572	3·8
April 1934—								
1	0·564	3·7	0·761	5·0	0·584	3·8	0·769	5.1
2	0·683	4·5	0·866	5·6	0·640	4·2	0·812	5·3

Table VII shows that the loss by evaporation during the day hours from the top-most layer of the soil is more or less recouped by absorption of water vapour from the air during the night. The loss as well as the gain at the level of the office terrace are somewhat larger than those at the ground level. This is due to the larger air movement at the higher level. On certain days the absorption is greater than evaporation or *vice versa*. These minor variations are due to non-uniformities of weather, *e.g.*, sea breeze sets in late in the evening on some days, thereby making more moisture available for absorption during night. Similarly strong winds during the day hours on certain days give rise to greater evaporation than absorption. Cloud in the afternoon of certain days would also inhibit evaporation as the dura-

tion of solar heating will be diminished. The effect of variable weather on the moisture balance at the soil surface will require further careful analysis.

The conclusions suggested by Table VII may also be arrived at by finding out the percentage moisture content of soil samples taken at sunrise and at sunset from the surface of an open, bare plot representative of the local conditions. Six samples were taken at the above times and the percentage moisture content estimated in the usual manner. Table VIII shows the results obtained for a few days in March 1934. The actual moisture content of course varies from day to day but, invariably the soil is richer in moisture at sunrise than at sunset, thus confirming the results shown in Table VII. The values of evaporation or absorption in Table VII are larger than in Table VIII presumably because in the former case the soil had been sifted and particles were uniform and small.

TABLE VIII.

Percentage of moisture in surface soil samples at sunrise and sunset.

Date March 1934	Mean percentage moisture content at sunrise	Mean percentage moisture content at sunset	Loss by evaporation during day (per cent.)	Gain by absorption at night
8	5·6	2·5	3·1	..
9	4·7	1·7	3·0	2·2
10	4·8	3·4	1·4	3·1
11	6·8	2·5	4·3	3·4
12	5·8	3·5	2·3	3·3
15	2·6	1·5	1·1	..
19	6·9	4·5	..	2·4
20	7·5	5·3	2·2	3·0
23	5·8	4·4	..	1·4
24	5·5	4·1	1·4	1·1

The data presented in the present paper also show that in taking samples from the upper layers of the soil for measurement of soil moisture, due consideration has to be given to the time of day at which the samples are removed from the ground.

Further work with other types of soils is in progress. The physical process by which different air layers near the ground contribute to the moisture absorbed by the soil surface and the hourly variation of evaporation or absorption in the case of different soils will be discussed in a later paper.

In conclusion, the authors desire to express their thanks to the Director-General of Observatories for facilities given at the laboratories of the Meteorological Office, Poona, and to the staff of the Agricultural Meteorological Observatory who recorded the hourly observations discussed.

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PETALODY IN COTTON.

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(With Plates LXII and LXIII.)

Introduction.

In the course of floral observations that were being made at the Cotton Breeding Station, Coimbatore, two types of petalody in cotton were noticed. One of them was observed in a bulk crop of Karunganni (*Gossypium indicum*) and described by the junior author [Sankaran, 1931]. The flower buds in this plant were stouter and smaller in size. When they opened, all the stamens on the lower three-fourths of the staminal column were completely transformed into full petaloid structures (Plate LXII, fig. 1), while a few of the upper stamens exhibited various transitional stages in the metamorphosis of a normal stamen to a petal. In many cases anther sacs were found suppressed or deformed on the margins. Microtome sections of a few specimens revealed that these petaloid structures were the result of the dilation of filaments (Plate LXII, fig. 2). At the very summit of the staminal column were present about half a dozen normal stamens containing fertile pollen grains. The stigma was characterised by the disherent lobes and entire absence of hairs on the stigmatic surface except for a very few long, white, filamentous hairs just at the tips. The plant was in all other respects normal.

The second type* of petalody occurred in a strain extracted from a natural cross between *Gossypium cernuum* and *Gossypium indicum* and appeared to be distinctly different from the one described above. Though many of the stamens on the lower half of the column were metamorphosed into petals, they were less distinct being considerably smaller in size and paler in colour. The anthers at the top were whitish and contabescent. The stigma was thin and slender with coherent lobes and a few rows of hairs on the ridges (Plate LXIII). The plant was stunted in appearance and did not appear to be a modification of the first type caused by variations in the intensity of development.

* This was first detected in the field by Mr. R. Balasubramania Ayyar.

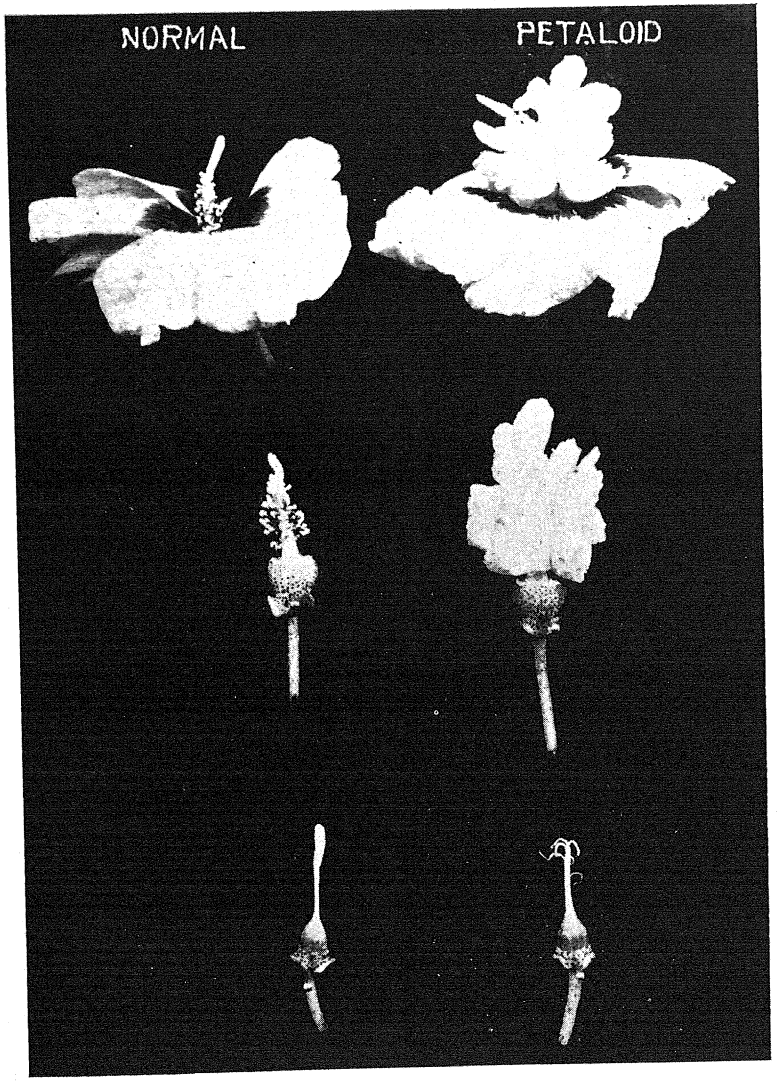


Fig. 1. Showing normal and petaloid flowers in cotton.

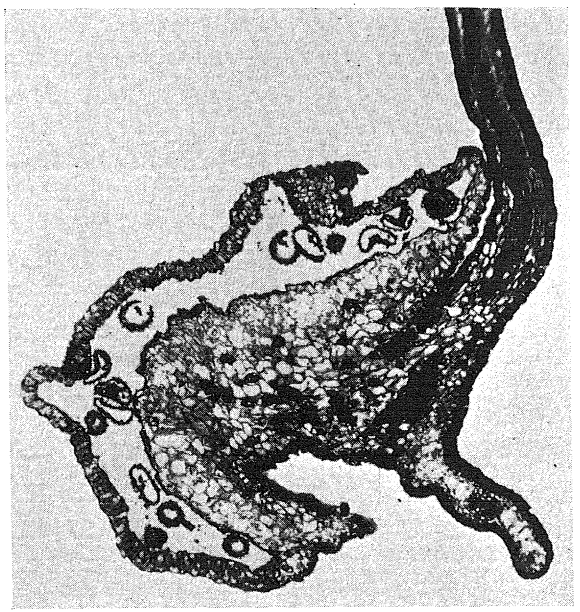
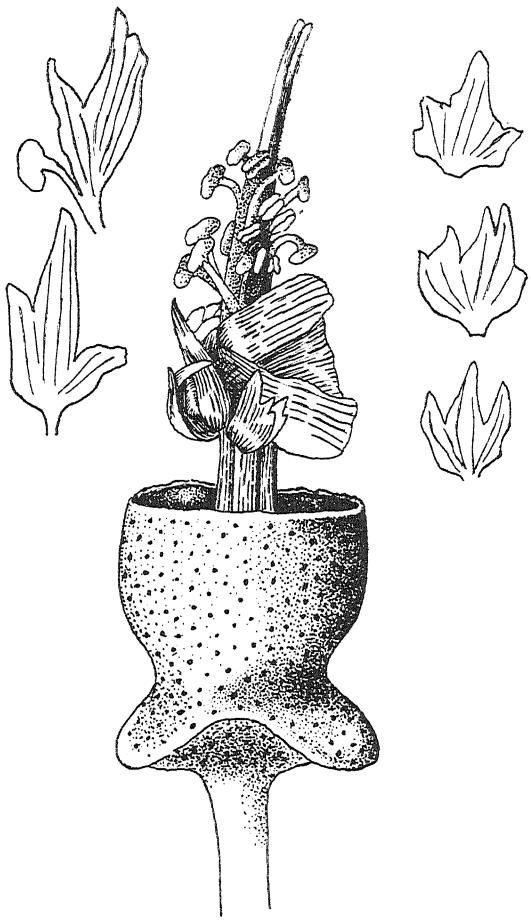


Fig. 2. Microtome sections of petaloid structures.

PLATE LXII.



Stigma with coherent lobes and hairs on the ridges.



As these types of floral abnormality have not so far been recorded in cotton, it was thought desirable to investigate if they are heritable and if so, their mode of inheritance. The results obtained so far are presented in this paper.

Previous work.

The inheritance of petalody was first investigated by Saunders [1910-11] in Petunia and later by Miyaki and Imai [1928] in the Japanese Morning Glory. The former worker obtained different ratios such as 3 : 1 in 9 families, 9 : 7 in 16, and 2 : 1 in 2. These led her to conclude that more than one factor were concerned in determining singleness and doubleness and that the proportion of germ cells carrying singleness and doubleness varied in different individuals. In a later publication [Saunders, 1928] she says "singleness is due to the presence of two factors **X** and **Y**; in the absence of either or both, the flower is double. In the true breeding singles, **X** and **Y** are carried by pollen and ovules in the linked condition (**X Y**). The Japanese workers found petalody to be recessive and influenced by a single factor.

Experimental work.

The flowers on these plants were selfed but no bolls were got out of them as expected. Their stigmas were dusted with pollen from different species of cotton only to get the same result. In the case of the second type there was absolutely no pollen and it was not possible to use it even as a male parent. These factors have completely precluded us from following its mode of inheritance. On the other hand in the first type a few pollen grains present at the top of the column were fertile and these were dusted on to the stigmas of emasculated normal flowers of Karunganni (*G. indicum*) plant. Out of 40 pollinated flowers only 8 bolls were obtained. Their seeds were sown during the season 1931-32 and 31 F_1 plants were raised. All the flowers borne by these plants were quite normal. The selfed produce from 5 of these plants was sown in 1932-33 for the study of F_2. In all of them there was sharp segregation into normal and petaloid plants. When counts were taken in each culture, a monohybrid ratio was obtained, indicating that a single genic difference was involved in the production of petalody (Table I).

Table I.

Family No.	1	2	3	4	5	Total	Expected
No. of normal plants	19	5	13	34	17	88	84
No. of petaloid plants	4	3	5	9	3	24	28
Total	23	8	18	43	20	112	112

[a] (3 : 1)=0·762. Deviation not significant.

Though the differences between expected and the observed are not statistically significant, the number of petaloid plants is lower than expected in most of the families. It is likely that the viability is less in seeds that are to produce petaloid plants.

No marked differences were perceptible between the heterozygote and homozygote in the normal phenotype. All the flowers borne by the petaloid plants in this generation exhibited all the characteristic features of the original parent (Type I). In every case there was the association of dismemberment of the stigmatic lobes and absence of papillae on the lobes. None of the numerous flowers produced by the petaloid plants developed into bolls, except for solitary boll which contained 18 well-developed seeds.

The seeds from ten of the phenotypically normal plants in F_2 generation were sown separately in 1933-34 for the study of F_3. When they were classified, the following results were obtained (Table II).

TABLE II.

Family No.	1 : 1	1 : 2	1 : 3	1 : 5	1 : 6	2 : 1	2 : 3	2 : 4	Total	Expected
No. of normal plants .	18	15	11	20	51	20	25	31	191	181·5
No. of petaloid plants .	6	4	4	5	10	4	13	5	51	60·5
Total .	24	19	15	25	61	24	38	36	242	242

χ^2 (3 : 1)=1·990. Deviation not significant.

Family No.	1 : 4	2 : 2	——
No. of normal plants .	24	35	Pure normals breeding true.
No. of petaloid plants .	*nil*	*nil*	
Total .	24	35	

Eight of the families have segregated into a simple 3 : 1 ratio while two produced only normal flowers. These figures reaffirm the hypothesis made from the F_2 data.

The 18 seeds obtained from the single boll on a petaloid plant of F_2 were sown in 1933-34 and all the plants raised manifested petalody with disherent and smooth stigmatic lobes. Surprisingly enough, even here only one boll contrived to set and produced 3 good seeds.

A study of behaviour of this character in different genetical background as well as the effect of the lethal gene on the development of the embryo and lint hairs, and on the distribution of seed and lint weights is in progress.

The pair of genes responsible for the petalody may be represented by F^{pd} and f^{pd}.

Discussion.

It is evident from the above data that petalody in cotton behaves in the same way as that found by the Japanese workers in the Morning Glory and is produced by a single genic difference. That it segregates in a clear-cut fashion from the normal is not surprising, as the crosses studied were made only with the species from which the mutant arose. The fact that it behaves as a recessive is also in complete accordance with the general behaviour of lethal genes mutating in the normal type. The non-setting of bolls in the petaloid plants seems to be due to the absence of stigmatic hairs; for, the ovules and the conducting tissues in them have been found to be quite normal. The defective external condition of the stigma does not seem to permit the pollen to be kept in position till it germinates and sends its tube into the conducting tissues.

The setting of a solitary boll in the F_2 and another in the F_3 petaloid plants calls for an explanation. As parthenogenis was observed in *G. indicum* at the Cotton Breeding Station, Coimbatore [Balasubramaniam, 1931], it was first considered that the two bolls in question might be of parthenogenetic origin. But the simultaneous production of 18 well-developed seeds in a boll normally containing 20 to 25 seeds would however seem improbable without the intervention of male gametes. Very probably due to some disturbance in the genic balance or to some mechanical accident to the stigma, conditions favourable for the germination of the pollen grains on the particular stigma might have been produced.

In the second type of petalody, the non-development of seeds in spite of the presence of stigmatic hairs and the dusting of fertile foreign pollen seems to be due to the defective condition of the ovules.*

* Since writing this note one solitary boll was obtained when crossed with Karunganni (*Gossypium indicum*).

The authors are thankful to Mr. J. B. Hutchinson for perusing the manuscript.

SUMMARY.

Two types of petalody in cotton are described—

(*a*) In one case a few fertile pollen grains are present but the stigma is devoid of hairs and the lobes become separate. The ovules seem to be fertile.

(*b*) In other case, the degree of petalody is weak and the anthers are entirely contabescent. The stigma is coherent and bears hairs, though less than the normal. Despite these favourable features no bolls had developed even with artificial pollination. It is presumed that the ovules are functionally sterile.

2. The pollen from the first type was dusted on to the stigmas of normal flowers. In F_1 and subsequent generations the normal condition of the flower was dominant. This character segregated in a monohybrid ratio indicating thereby that petalody is caused by single genic difference which is denoted by F^{pd} and f^{pd}.

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DISEASES OF *ELEUSINE CORACANA* GAERTN. AND *E. AEGYPTIACA* DESF. CAUSED BY SPECIES OF HELMINTHOSPORIUM.

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(With Plates LXIV-LXVII and seven text-figures).

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I. Introduction.

Helminthosporium nodulosum B. et C. was first recorded on *Eleusine indica* Gaertn. and described later on by Berkley [1857, 1875]. *H. nodulosum* has also been recorded on *E. indica* from America [Drechsler, 1923], Philippines [Sydow, 1913], Japan [Nisikado, 1929], and on *E. coracana* from Uganda [Snowden, 1927].

In India, *H. nodulosum* has been found on *E. coracana* from Coimbatore and Wynaad (Madras Presidency), Pusa and Chota Nagpur (Bihar), Almora and Kumaon (United Provinces), and also on *E. aegyptiaca* from Pusa. *H. leucostylum* Drechs. has also been recorded on *E. indica* and *Ergrostis major* [Drechsler, 1923] from the United States of America and on *E. indica* from South Africa [Smith and Putterill, 1933]. A species somewhat similar to *H. leucostylum* was isolated from *E. coracana* at Pusa and is referred to in the text as *Helminthosporium C. H. tetramera* [McKinney, 1925] originally described on wheat was also found on *E. aegyptiaca* but has not been included in this study.

II. Morphology and parasitism of *H. nodulosum* B. et C.

(*i*) *Symptoms.*

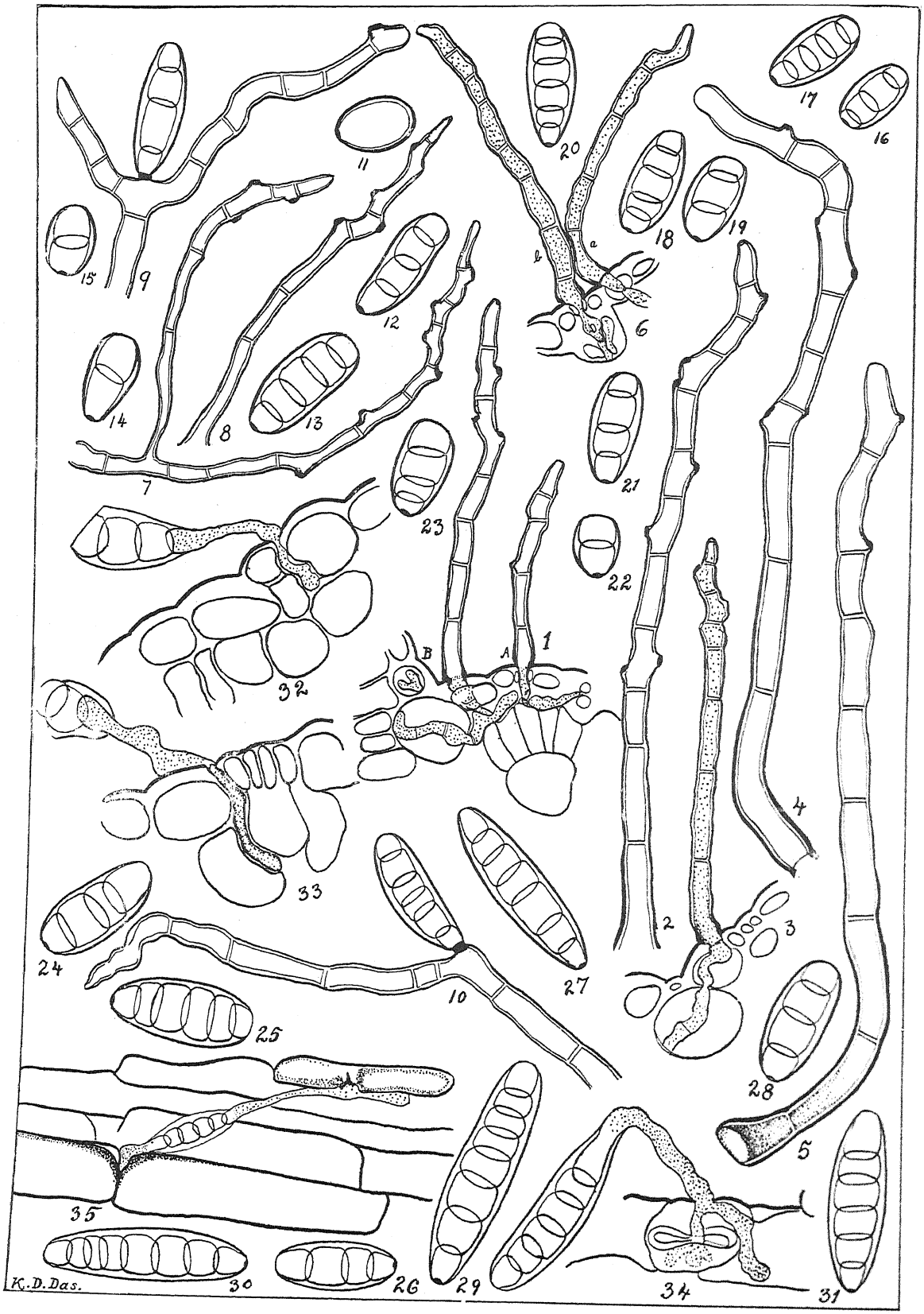
The fungus is a virulent parasite and may attack the root, stem, leaf-sheath, leaf, spike, spikelet, and often kills plants in the seedling stage. The disease makes its appearance on both sides of the leaf as small oval mineral brown spots, which gradually elongate parallel to the axis of the leaf and later become dark chocolate brown. The mature spots generally are about 10 × 1.5 mm., and by their coalescence large irregular patches are formed. The formation of stripes or shredding of leaves does not take place as it does in *Helminthosporium C.* referred to later. Heavily affected leaves wither prematurely, and the grain yield is consequently reduced. The spots on the leaf-sheath are not well defined. They are much larger in size and of a chocolate brown colour, and are commonly seen at the junction of the leaf blade and the leaf-sheath.

The spots on the stem are oval to oblong and of a sepia colour. The cortical tissue is invaded and at times collapses, resulting in the death of the plant above the spot. " Foot-rot " or " root-rot " is also caused by this fungus, specially in fields where unhealthy conditions prevail.

In a healthy inflorescence the spikelets have light chestnut-coloured seeds surrounded by pale yellow palæ which are wide open, but the diseased spikelets have grey palæ which remain closed, while the majority of the seeds are smaller, lighter, shrivelled and darker in colour.

In severe cases of attack grainless spikes and spikelets result. Healthy spikes as they mature become recurved, but in diseased heads they remain straight and widely separated at the end. In very humid weather the diseased inflorescences are of a sooty or olivaceous green, which is due to the superficial growth of the fungus on rachis, spikelets, and rachillas. The stalk bearing the inflorescence often shows a brownish spot which extends to a considerable length, and the inflorescence may hang down from that point.

PLATE LXV.



(For explanation see page 974.)

PLATE LXIV.



(For explanation see page 973.)

In diseased grains the glumes, the palæ, and the grain coat had hyphæ on the surface which when placed on culture media gave rise to colonies of *H. nodulosum*. These hyphæ on the glumes were thick-walled with small cells running longitudinally and on the palæ were aggregated into small hyphal masses. These hyphæ were also found on the warty surface of the grain coat. The conidia and the mycelium of the fungus on these diseased parts and grains were found to remain in a living condition for at least two years. These facts show that the disease is seed-borne and the initial infection comes from diseased grains. This accordingly suggests a method of checking the prevalence of the disease by seed treatment.

The time of infection and weather conditions are the chief factors which determine the severity and the spread of the disease. If the spikes become infected after the hard coat of the grains is formed, there is no diminution of the grain. On the other hand, if infection is earlier and the weather humid and cloudy, the fungus spreads rapidly, enters the floral parts and young grains, and results in a sterile spike. Cases are, however, observed in the fields where one spike is entirely healthy while the others are diseased, or in the same spike a part may be healthy and the remainder infected. Plate LXIV, figs. 1-5 show details of symptoms on the host plant.

(*ii*) *Morphology of the fungus.*

The mycelium consists of septate, sub-hyaline hyphæ which ramify in the tissues of the host both inter- and intra-cellularly, haustoria being absent. The hyphæ are about 4μ in diameter and where they pierce the cross walls, they become very narrow and assume their former width on reaching the opposite side (Plate LXV, fig. 3). The hyphæ are found in all the diseased parts, even in sclerenchymatous and vascular tissues and pass from cell to cell in the sclerenchymatous tissue of the stem through the small thin-walled areas in their longitudinal walls. The mycelium bearing the conidiophores is of an olivaceous colour.

Conidiophores (Plate LXV, figs. 2, 4, 5) are formed on the central dead portion of the infected area, are stout, erect and rigid, arising singly or in clusters of two to seven from the stomata but rarely through the epidermis, and are also formed from the creeping hyphæ on the infected portion of the host. They are bulbous at the base, generally unbranched, are often much curved with prominent geniculations, dark brown to dark otter brown at the base with a hyaline to sub-hyaline tip. The apical cell is 3-5μ in diameter with a rounded hemispherical end. They have 4-18, usually 7-11 septa, the average being nine. In size they are 72-356×3·5-8μ, the average being 150·6×6μ (400 measurements).

The average width of the swollen basal cell is 12·7μ and the range 8-18μ. The number of conidia borne on each conidiophore is 1-11.

Conidia (Plate LXVI) are thick-walled, subcylindrical or ovate, rarely obclavate, slightly curved or straight, widest near the middle tapering towards both ends which are rounded off abruptly, and there is a conspicuous hilum. The apical cell is slightly lighter in colour with a hemispherical end. The tapering is more pronounced towards the apex. Typically the peripheral wall of one side is more curved, while the other is more or less straight, giving a characteristic shape to the conidium. When fully mature the conidia are of a light russet green or reseda colour, the end cells being paler with a narrow sub-hyaline zone at the extreme apex and a similar narrow zone at the distal end. They measure from 40-114× 11-21μ with 3-11 septa, not associated with constrictions, the average length, width and septa (800 measurements) being 67μ, 14μ, and 8 respectively.

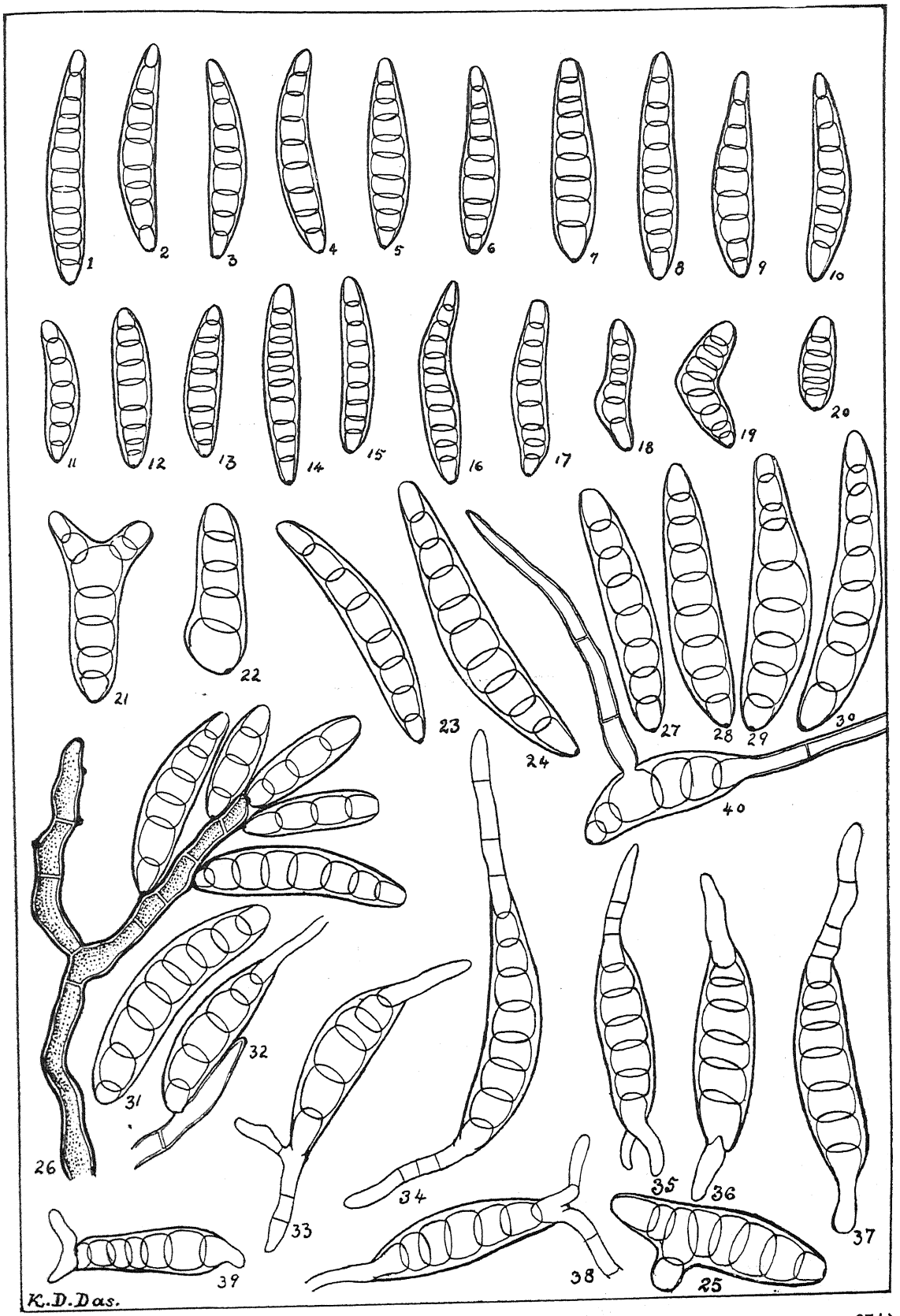
(*iii*) *Parasitism.*

(*a*) *Inoculation on the host.*—Single spore cultures of *H. nodulosum* from *E. coracana* were obtained from diseased specimens collected at Pusa and also from material obtained from Coimbatore, and were found to be identical. *H. nodulosum* was also isolated from *E. aegyptiaca* and was identical with that isolated from *E. coracana*. Series of inoculation experiments were carried out on *E. coracana* both in the field and laboratory on plants in different stages of growth with pure cultures of *H. nodulosum*. All the parts of the plants were inoculated either with spore suspensions in water or with spores and mycelium, and the inoculated plants were kept covered with bell jars or in moist glass chambers. The results of the infection experiments are summarised in Table I which shows that the fungus is a virulent parasite capable of attacking all the parts of a host plant.

TABLE I.

Summary of the infection experiments carried out with H. nodulosum *on* Eleusine coracana.

Parts of the plant inoculated	Number of inoculations	Number infected	Number of controls kept	Controls infected
Leaf wounded (Both sides)	59	59	13	*Nil*
Leaf unwounded (Both sides)	100	94	35	,,
Leaf-sheath	20	20	10	,,
Inflorescence	38	36	16	,,
Rachis	14	14	4	,,
Stem	10	9	9	,,
Leaf axil	34	34	16	,,



(For explanation see page 974.)



The first sign of infection on the leaf was noticed within twenty-four hours as a minute brown spot which soon elongated and within four days produced conidiophores and conidia. After the infection had been established, the inoculated plants were removed into the open. It was observed that during the months of June and July though the infection took place, it did not spread, while during August and September when the temperature is comparatively lower and atmospheric humidity higher, infection was very readily taken and spread rapidly. In the field too the disease was more severe in these months than in June and July. It was further observed that the infection took place more quickly from between the leaf-sheaths and also from the upper surface of the leaves.

Inoculation on the inflorescences was done by spraying them with a suspension of spores in water and keeping the plants in moist chambers or wrapping them in moist and sterilized cotton wool. Here too the first sign of infection was noticed within twenty-four hours. After that period the plants were kept under natural conditions of growth. The infection was most severe when the experiment was carried out any time between the appearance of the inflorescence and the formation of grain, that is, at any stage before the grain coat had hardened, and resulted in the complete suppression of grain formation. Under favourable conditions inoculation of only one spike resulted in the rapid spreading of the fungus, superficially involving neighbouring spikes and spikelets which soon presented a bluish or mouldy appearance due to the abundant fructification of the fungus.

Seed of *Eleusine coracana* was disinfected with a solution of mercuric bichloride (1 in 1000) for five minutes and washed in several changes of sterile water. It was transferred to sterilised potato tubes filled to the narrow stem with Knop's solution and containing a little cotton wool. When the seedlings were two or three inches high, they were inoculated at various places, on the roots, coleoptile, and the growing region. Under these conditions all the inoculated plants took infection readily within twenty-four hours, and in three days' time they were killed. The controls in all the cases remained healthy.

Infection did not take place at 10°C., was slight at 37·5°C., and the optimum temperature was found to lie between 30°C. and 32°C. at which temperatures the first sign of infection was visible within twenty hours and the seedlings died within three days. The comparative data of the extent of disease produced at various temperatures are expressed as "infection rating", shown in Table II, representing the percentage of the total number of plants which were infected and also the degree of infection. The method of calculating infection rating is that adopted by McKinney [1923] and Mitra [1931].

TABLE II.

Effect of temperature on the infection of Eleusine coracana *seedlings with* H. nodulosum *after fifty-two hours.*

Temperature (°C.)	Number of plants	Infection rating
10	61	0·0
18	81	23·0
22	92	29·5
25	96	45·8
30	106	79·2
32	106	80·0
35	86	37·5
37·5	95	7·3

The effect of temperature on leaf infection was determined by inoculating cut shoots sprayed with a spore suspension in water and kept in flasks covered with bell jars at various temperatures. The results obtained were similar to those in seedling infection.

The spots appeared within twenty-four hours between 25°C. and 35°C. Those appearing at 30°-32°C. were like normal lesions produced on leaves in the field which in the season were growing at temperatures from 24-37°C. Spots appearing at 35°C. and above remained small. Inoculated plants after the establishment of infection when left in the open in the hot weather developed similar small spots. The germ-tubes often formed oval or oblong appresoria (Plate LXV, figs. 32-35), and infection took place through the stomata or directly through the epidermis. Usually it takes place between epidermal cells. The entrance of germ-tubes was often followed by a brownish discoloration of the neighbouring cells. Once within the epidermal cell, they grew in the tissue of the host both as intra- and inter-cellular hyphæ which after sometimes gave rise to conidiophores.

(*b*) *Cross-inoculations.—H. nodulosum* was cross-inoculated on a number of cereals with a view to determine the possible host range. The details are given in Table III. The numerator of each fraction gives the number of infections, the denominator the number of inoculations made.

TABLE III.

Cross-inoculation experiments on various hosts with H. nodulosum.

Host	Inoculations	Percentage infection	Host	Inoculations	Percentage infection
Triticum vulgare	4/40	10	*Zea Mays*	96/99	97
Avena sterilis	1/36	3	*Saccharum officinarum*	26/36	72
Hordeum vulgare	3/29	10	*Pennisetum typhoideum*	30/35	85
Panicum miliaceum	49/49	100	*Eleusine indica*	72/80	90
Panicum frumentaceum	78/94	82	*E. aegyptiaca*	94/96	98
Sorghum vulgare	47/49	96			

It is thus seen that *H. nodulosum* is capable of infecting a wide range of host plants in addition to the two other species of *Eleusine* commonly found in Pusa. *Zea Mays* and sorghum plants were affected with such severity that the young plants died, but the spots on the leaves of sugarcane remained very small.

III. MORPHOLOGY AND PARASITISM OF *HELMINTHOSPORIUM C.*

(i) Symptoms.

The disease makes its appearance earlier in the season but is soon over-shadowed by the more destructive one caused by the more rapidly growing *H. nodulosum*. The spots are formed on both the sides of the leaves, usually at the margin or tip, and they gradually spread longitudinally parallel to the leaf-veins (Plate LXIV, fig. 6). In the beginning they are saffron yellow to cadmium yellow without any defined outlines, and as they grow the central part becomes chocolate brown. Many spots usually run together and coalesce, covering the entire upper half or the margin of the leaf. Often the union of many spots results in long stripes with irregular margins. The central part becomes thin and grey, veins remaining prominently visible and the affected parts showing a pronounced tendency to split into numerous longitudinal shreds. The inflorescence is also attacked and the symptoms are very much like those caused by *H. nodulosum*.

(ii) Morphology of the fungus.

The mycelium consists of thin-walled, septate hyphæ, both inter- and intra-cellular, running into all the tissues of the host plant. There are no haustoria. The hyphæ are broader than in *H. nodulosum* and are hyaline even at the place at which conidiophores are given off.

Conidiophores are formed on the infected parts of the leaf, more abundantly on the upper than on the under surface. They are usually confined to stomata and emerge singly or in clusters of two to nine (Plate LXVII). They are sub-hyaline when young, and greenish white or light olivaceous when mature, hyaline at the tip and base with prominent geniculations, unbranched or producing short lateral branches. The basal cell is sometimes swollen and sub-hyaline, continued by a narrow lower part of the conidiophore which gradually becomes broader at the top. The tip is flat or anvil-shaped. The places representing the original attachment have prominent circular scars. Conidiophores are 28-112μ in length with an average of 68·9μ, with 2 to 8 cross walls associated with slight constrictions. The width in the centre is 4·5-6·5μ. The cross walls are inserted at intervals of 10-28μ. Conidia are borne at a distance of 18-40μ from the base, and the successive spores at intervals of 5·25μ.

Conidia are olive-green to light olive-brown in colour, obclavate, sometimes ovoid, a typically obovoid, widest at the second septum near a point approximately one-third of the distance from the base to the apex. The proximal portion tapers uniformly to a narrow apex and then is rounded off abruptly, the apex being hemispherical. The hilum at the distal end is of a dark colour, fairly conspicuous but not protruding. In size conidia are 18·0-78·8$\times$10·0-20·8μ, 1-8 septate, septa never associated with constrictions.

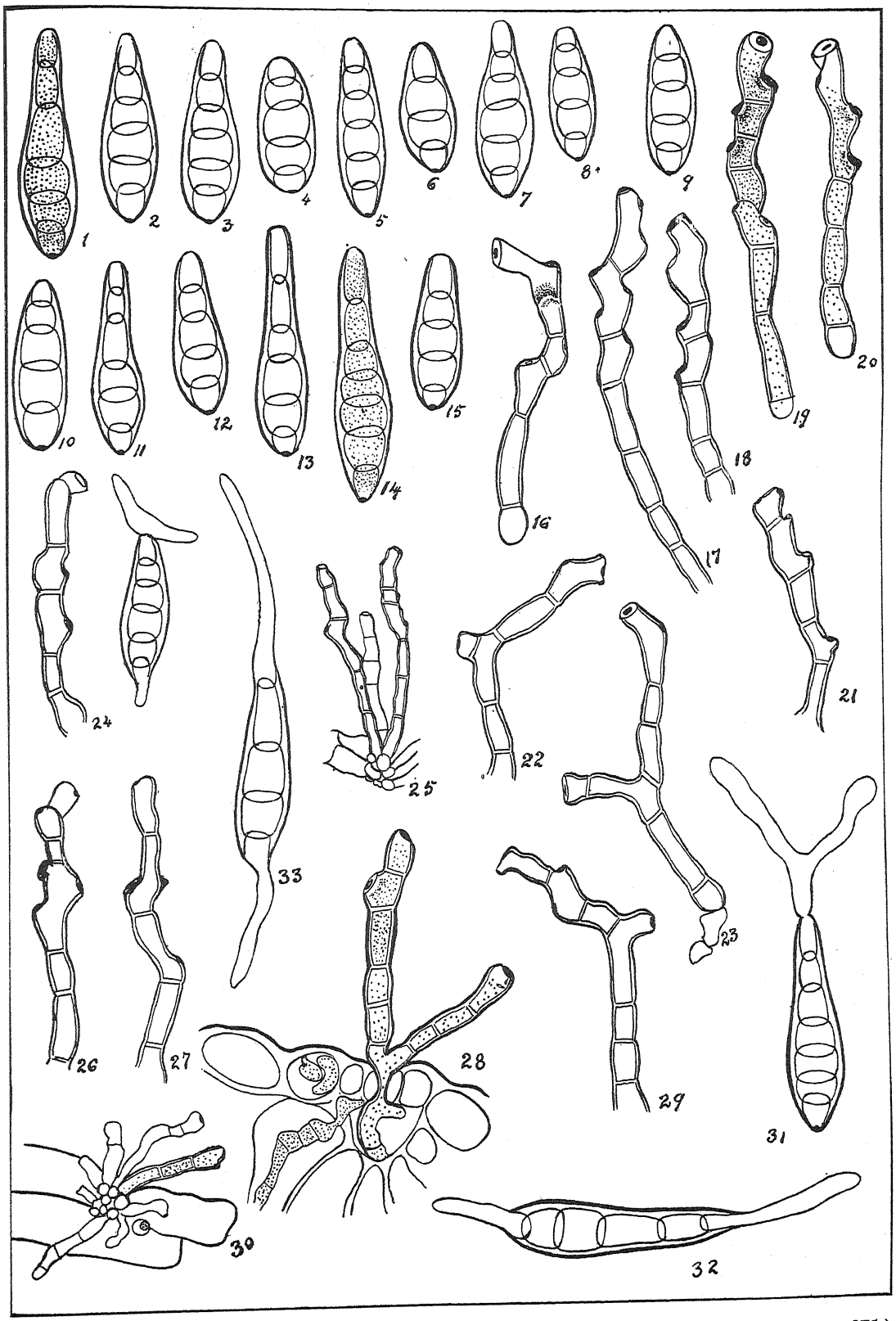
(*iii*) *Parasitism.*

(*a*) *Inoculation on the host.*—Series of inoculation experiments with single spore pure cultures were carried out both in the field and the laboratory on plants of *E. coracana* of different ages, and the results show that the fungus can attack every part of the host plant and at different stages of its growth. The details are summarised in Table IV.

TABLE IV.

Summary of the inoculation experiments carried out on E. coracana *with* Helminthosporium C.

Parts of the plant inoculated	Number of inoculations	Number took infection	Number of controls	Number of controls infected
Leaf both sides	128	116	35	*Nil*
Leaf-sheath	28	24	10	,,
Inflorescence	40	36	12	,,
Seedlings	40	32	12	,,



(For explanation see page 975.)

The first sign of infection became visible after forty-eight hours in the form of a yellow discoloration of the inoculated portion of the leaf. Definite spots did not form as in the case of *H. nodulosum*. The percentage of successful inoculations was lower than in *H. nodulosum*, and the spots, which took a longer time to appear, increased very slowly. These facts show that *Helminthosporium C.* is comparatively a weak parasite.

(*b*) *Cross-inoculations.—Helminthosporium C.* was cross-inoculated on a number of cereals and found capable of infecting many. The details are given in Table V. The numerator of each fraction denotes the number of infections, the denominator the number of inoculations made.

TABLE V.

Summary of cross-inoculation experiments on various hosts with Helminthosporium C.

Host	Inoculations	Percentage infection	Host	Inoculations	Percentage infection
Zea Mays . . .	26/30	86·6	*Eleusine indica* . .	10/18	55·5
Saccharum officinarum .	0/18	0·0	*Eleusine aegyptiaca* .	16/18	88·8
Panicum frumentaceum	22/31	70·9	*Pennisetum typhoideum*	7/12	58·3
Sorghum vulgare . .	20/22	90·9			

The fungus is thus capable of infecting all the hosts on which it was tried except sugarcane. It is a weak parasite as compared to *H. nodulosum* and the spots formed increase very slowly in size. When only mycelium was used as a source of inoculum, infection took place much less readily.

IV. STUDIES UNDER CONDITIONS OF ARTIFICIAL GROWTH.

The following strains of Helminthosporium were used in the study :—

1. *H. nodulosum* isolated from *E. coracana.*
2. *H. nodulosum* isolated from *E. aegyptiaca.*
3. *Helminthosporium C.* isolated from *E. coracana.*

The purity of each strain was assured by taking single spore cultures, and all stock cultures were maintained on Brown's synthetic agar and kept at 20°C. Cultures used were always of the same age and from the same medium.

(i) Growth on various media.

The growth characters of all the three strains were studied on a large number of media. The two isolations of *H. nodulosum* agreed in cultural characters and morphological details on all the media tried and are therefore considered to be identical.

H. nodulosum grew best on corn-meal agar, barley-meal agar, rice-meal agar, Brown's synthetic starch agar, Richards' solution agar, and prune juice agar. The best aerial growth with poor sporulation was obtained on French-bean agar, Winogradsky's glucose-peptone agar, Richards' solution agar and Eleusine leaf-extract agar. Copious sporulation with scanty aerial growth was obtained on rice-meal agar, corn-meal agar, Brown's synthetic starch agar, prune juice agar and *N*/50 to *N*/100 Richards' solution agar. Brown's synthetic agar gave a poor aerial growth and sporulation. The fungus grew well on cooked rice and cooked *E. coracana* grains, producing a good deal of mycelial clumps and sclerotial bodies though sporulation was very poor on these media. Moist sterilised wheat straw, sterilised green leaf and straw of *E. coracana* formed suitable media for copious spore formation.

The colour of the colonies differed on various media. It was olive-green on corn-meal agar, rice-meal agar, prune juice agar, and Brown's synthetic starch agar; greyish on Richards' solution agar, Winogradsky's glucose-peptone agar, French bean agar, Brown's synthetic agar ; and almost white on Eleusine leaf-extract agar. On cooked rice grains the fungus exhibited vivid coloration (brownish to greenish), depending on the amount of water and the position of the mycelium. When the amount of water was less, numerous white or pinkish mycelial clumps were seen along the glass edge and the mycelium between the grains showed a brownish tinge. On the other hand when the water is more, the number of mycelial clumps was considerably reduced and the fungus showed a greenish coloration.

On most of these media zonation was obtained in plates kept alternately in light and darkness. Zonation was best on Brown's synthetic agar, corn-meal agar, rice-meal agar, Brown's synthetic starch agar, etc., while it was not so good under similar conditions on French-bean agar and barley-meal agar and entirely absent on Richards' solution agar.

Helminthosporium C. was found to be a slow growing fungus though it grew well on most of the media tried. On Brown's synthetic medium the colony consisted mostly of white aerial mycelium which at a temperature of 18°C. to 20°C. showed a tendency to twine into strands specially near the margin. The sporulation was poor. At higher temperatures and with age the colony matted down giving the appearance of a thin, dull glazed layer. On Brown's synthetic starch agar

the colony was white showing marginal strands of hyphae at 16°C. to 18°C. The mycelium showed a marked tendency to a submerged growth of a violet colour. Sporulation was fair on this medium and the conidiophores were borne on the surface of the medium on submerged mycelium. The fungus grew well on Richards' solution agar giving a pinkish colony. There was little spore formation and with an increase of temperature the colony became felty and of a deeper tint. The growth and sporulation were fair on oat-meal agar, while on moist sterilised straw of *E. coracana* the aerial growth was little and the sporulation was copious. The fungus grew well on cooked rice giving good aerial growth of a pink colour and scanty spore formation.

(*ii*) *Spore germination.*

Spores of *H. nodulosum* and *Helminthosporium C.* germinated readily in distilled water, but when placed in some nutrient solutions such as Coons' synthetic solution, five per cent. solution of glucose, or of cane sugar, the spores germinated more quickly. It was noticed that the spores which developed under different environmental conditions showed differences in germination, and in order to avoid irregularities in comparative studies spores eight days old from cultures on sterilised green leaves of *E. coracana* (at 30°C.) were taken. The mode of germination agreed with those of other species of *Helminthosporium* as *H. sativum* P. K. and B., *H. sacchari* Butl. [Butler, 1918] (Plate LXV, figs. 33-40). Generally the end cells of spores germinated but on rare occasions specially in spores formed at higher temperatures such as at 32°C. on rich starchy media germination took place more frequently from intermediate cells. When a spore broke, the cells at the broken end germinated. This shows that all the cells of the spores possess the capacity to germinate though usually it is restricted to the end cells where the peripheral wall is thinner. The conidiophore cells also occasionally function as conidia by sending out a germ-tube. The growth of germ-tubes with unusual bends in *H. leucostylum* Drechs. as noticed by Drechsler [1923] was not observed in *Helminthosporium C.*

(*a*) *Temperature.*—The optimum temperature for spore germination in *H. nodulosum* and *Helminthosporium C.* was 30°C. to 32°C. and 28°C. to 30°C. respectively. The temperature range for germination was from about 10°C. to 39·5°C. in the case of *H. nodulosum* and from about 10°C. to 38·5°C. in the case of *Helminthosporium C.*

(*b*) *Humidity.*—The minimum humidity required for the germination of spores of *H. nodulosum* was determined according to the method of Lesage [1895]. The principle involved is that the saturation of air above a given solution of sodium chloride varies inversely with the concentration of the salt dissolved therein and the humidity is said to remain constant though the temperature may vary. Moisture-

free sodium chloride was taken and solutions containing 4·5 to 25·0 grms. per 100 c.c. of water were prepared and 25 c.c. of the solution was put in dishes 6 cm. × 3·5 cm. A drop of distilled water containing a spore suspension was spread on the underside of each of the lids and dried in 'vacuo' over calcium chloride. The lids were then placed over the dishes and sealed with a mixture of paraffin and vaseline. All the dishes were placed in an incubator running at a constant temperature of 30°C. The results obtained are given in Table VI.

TABLE VI.

Germination of the conidia of H. nodulosum *at different relative humidities at 30°C.*

Grms. NaCl per 100 c.c. water	Relative humidity	Germination percentage in hours			
		6	22	46	70
0·0	100·0	32·5	83·6	91·1	94·6
4·5	97·3	23·0	63·8	78·3	82·5
7·0	95·7	15·6	39·3	54·6	61·1
10·0	93·4	9·8	19·1	26·0	36·0
12·0	92·7	6·8	11·0	15·1	20·1
15·0	91·0	0·0	1·0	4·0	5·8
16·0	90·0	0·0	0·0	0·0	0·0
25·0	84·9	0·0	0·0	0·0	0·0

The best germination was obtained at 100 per cent. humidity, and it gradually decreased upto 90 per cent. when there was no germination. The atmospheric humidity at Pusa from August to October remains fairly high, and germination of conidia is possible during these months. The disease appears and spreads more readily during these months, probably due to favourable conditions for the germination of conidia.

(*c*) *Plant tissues.*—Several investigators have observed that the presence of the host tissue affects the spore germination of various pathogenes. The effect of the host tissue on the spore germination of *H. nodulosum* was tested by floating conidia over distilled water in watch glasses of uniform size. To these watch glasses seedlings about 1-2 cm. long thoroughly washed in distilled water were put. In another set cut pieces of seedlings were placed and the controls contained distilled water only with spores. The germination percentage and the length of germ-tubes

of floating spores were measured after a fixed interval of time. The results obtained are given in Table VII which represents an average of 200 germ-tubes in microns.

TABLE VII.

Germination of conidia of H. nodulosum *in distilled water and with the addition of tissues of various plants after two and a half hours.*

Plant tissues	Length of germ-tubes in microns	Germination percentage
Control	60	66
E. coracana	130	95
E. coracana (boiled leaf)	60	65
Phaseolus leaf	130·5	97
Zea Mays leaf	128	96
Sorghum vulgare leaf	125	90
Pennisetum typhoideum leaf	130	96
Paspalum scrobiculatum leaf	122	90
Panicum miliaceum leaf	104	90

Conidia germinated uniformly in dishes containing host tissues in suspension, developing long, branched and vigorous germ-tubes, while on the contrary spores germinating in water only usually produced slender, mostly unbranched and short tubes. Within the short period of observation it was noticed that the spores in dishes containing host tissues almost always germinated at both the ends while those in control dishes often germinated from one end only. Further it was observed that in the controls there was a decidedly higher percentage of germination of spores floating on the surface (66 per cent.) than in the case of the submerged ones (3 per cent.). On the other hand little or no difference could be noticed in the percentage of germination of floating or submerged spores on the addition of the host tissues. Christensen [1926] also made similar observation for spores of *H. sativum*. It appears that host tissues exerted some chemotropic influence which was evidenced while observing the direction of germ-tubes showing positive chemotropic reaction. The addition of cut seedlings gave a higher percentage of germination than the addition of entire seedlings, and in both cases the average length of germ-tubes was greater than in the control. The stimulating effect was not specific for any particular plant, nor was the vigour of the germ-tubes affected

with the susceptibility of the plant. Sterilised green leaf did not stimulate germination.

Leach [1923] working on the germination of the spores of *Colletotrichum lindemuthianum* (Sacc. and Mag.) Br. and Cav. observed that the tissues of plants other than the host stimulated germination. Noble [1924] showed that even the unwounded seedlings of non-susceptible plants stimulated the germination of the spores of *Urocystis tritici* Koern. Christensen [1922] working on *H. sativum* determined the effect of various non-susceptible plant tissues on the germination of conidia and found that " apparently the stimulatory agent is not specific ". He also noticed that although the percentage of germination of conidia of *H. sativum* was usually not reduced by the presence of tissues from non-susceptible plants, the vigour of germ-tubes was usually greater in the presence of tissues from susceptible plants. The fact that boiled leaves failed where fresh leaves succeeded in stimulating the germination to a marked degree shows that stimulating substances are destroyed by heat. The addition of unwounded tissues also stimulates germination to some extent. This might be due to the exosmosis of nutrient substance in the 'infection drops' as shown by Brown [1922]. He noticed that *Botrytis* spores germinated readily in 'infection drops' on the surface of the host tissue but poorly in distilled water.

(iii) Macroscopic growth characters.

It is a well-known fact that most fungi exhibit growth variations on different culture media and on the same medium under different environmental conditions. Dosdall [1923], Christensen [1922, 1926] and Stevens [1922] have shown this in the case of *H. sativum*. Mitra [1931] in his extensive studies on various species of Helminthosporium on cereals and sugarcane has confirmed the observations of the previous workers. He has further shown that growth under different environmental conditions such as higher temperatures markedly affects the morphology of the fungus, so much so that the fungus can be mistaken for a different species. He further attributes to this fact the differences in the morphological characters of a fungus at different times of the year or as given by different authors from countries under different climatic conditions (*e.g., H. sacchari* Butl.). The changes, however, are not of a permanent nature, they are only phenotypic. Permanent changes are caused by mutation or saltation.

A comparative cultural study of *H. nodulosum* isolated from *E. coracana* and *E. aegyptiaca* was made, and in a few experiments *Helminthosporium* *C.* from *E. coracana* was also included. The experiments were carried out on Brown's synthetic agar, Brown's synthetic starch agar, and on Richards' solution agar. In

this study it was noticed that all the three strains behaved alike regarding the production of aerial mycelium, the development of colour, the formation of sclerotial bodies and zones. The changes in macroscopic characters were influenced by the quantity of nutrient medium per plate, the nature of the medium, temperature, humidity, light and darkness, the acidity or alkalinity of the medium, and by inhibitory influences. Taking these points into consideration, the medium was prepared at one time and was poured in equal quantities into uniform Petri dishes. All the plates after inoculation were kept under identical conditions and experiments were repeated to confirm the observations.

Aerial mycelium.—Temperature plays an important part in the aerial growth of the mycelium. The greatest development was at the optimum temperature for linear growth. Better development of aerial mycelium took place in cultures exposed alternately to light and darkness than those kept in constant darkness. Further, the development was more when the cultures were kept at alternating temperatures than at a constant temperature in darkness. At higher temperatures the colony remained small, compact and felty. In all the strains in the neighbourhood of the optimum temperature for surface growth, especially in rich media, small localised patches appeared on the surface of the colony, differing markedly from the parent colonies. The quantity and richness of a medium affected the development of aerial mycelium. With the same medium under optimum conditions of growth the amount of aerial mycelium varied with the amount of the medium in the plate, that is, the thicker the medium the denser the growth, and *vice versa*. On Coons' agar and Richards' solution agar it was observed that with successive dilutions there was a progressive reduction in the amount of aerial mycelium. Humidity of air also influenced the development of aerial mycelium which was most pronounced at a humidity between 80 and 92 per cent. In the presence of certain foreign organisms the formation of aerial mycelium was stimulated while others tended to make the colony grow submerged in the medium.

Of the three strains under consideration, *Helminthosporium C.* produced scanty aerial mycelium, whereas both the strains of *H. nodulosum* were alike in all respects and produced abundant aerial growth.

Colour.—Colour depends on the nature and the quantity of nutrient medium. It was noticed that the colour of the colony of *H. nodulosum* grown in plates with thinly poured Brown's synthetic starch medium was much darker than that of those grown on thickly poured plates. The colour of the colony also depends upon temperature, light relations and humidity. Cultures of *H. nodulosum* growing in the absence of light were darker in colour while in light there was often observed a pinkish tinge. *Helminthosporium C.* did not respond much to the environmental factors so far as colour development was concerned.

Similar variations in colour were noticed when cultures of *H. nodulosum* were kept at different temperatures. In *Helminthosporium C.* a marked change in colour was not observed and the colour in all the cases remained white or pale lilac-rose for aerial mycelium.

Colour changes may also be due to the humidity of the air and media. Cultures grown in Petri dishes hung upside down over various concentrations of sulphuric acid in closed vessels differed in colour. The variation in the humidity of the medium itself is able to induce colour variations. Cultures grown on rice grains autoclaved with different quantities of water exhibited difference in the colour of the mycelium. Similarly, colonies of foreign organisms which inhibited growth usually gave a dark grey colour to the edge of the growing colony opposite to it.

Zonation.—The three forms of *Helminthosporium* produced zones or concentric rings under certain conditions. Zonation was found mostly due to differential growth and structural characteristics of colony growth, such as, variation in the amount of aerial mycelium, density of spore massing and variation in the colour of the aerial mycelium. Zonation has been attributed to various causal agencies, such as, light, temperature relations, resting periods, staling products, mycelial crowding, alkaline medium and variation in the amount of food. Stevens and Hall [1909] have emphasized on the fact that zoning may be induced by the fluctuation of the foregoing external factors but it is largely dependent upon the fungus. Bisby [1925] in his review of the literature on the subject has stated that different fungi give variable results.

Though the number and sharpness of the zones in both *H. nodulosum* and *Helminthosporium C.* could be influenced by varying conditions, each form showed a difference in its capacity for zone formation. While *H. nodulosum* readily formed many zones, *Helminthosporium C.* under similar conditions formed only a few. The latter was also found to respond slowly to most of the media tried.

With regard to *H. nodulosum* from *E. coracana* and *E. aegyptiaca* the number of zones differed in different media. They were most sharply defined on rice-meal agar, on corn-meal agar and on Brown's synthetic agar. No zonation was found on normal Richards' solution agar but it did occur on agar with a less concentrated solution. Plates of all the media tried were kept in continuous darkness and in continuous artificial light produced by a 50 c. p. Osram gas-filled lamp, but no zonation occurred in either condition. When these plates were subsequently exposed alternately to day light and darkness, zones appeared in the new growth but none in the old growth. *Helminthosporium C.*, on the other hand, produced zones only on Brown's synthetic agar, rice-meal agar and prune-juice agar in alternating day light and darkness, but under no other condition.

Temperature also plays an equally important part in zone formation. This fact has also been noticed by Christensen [1926] in the case of *H. sativum*. Bisby [1925] obtained similar results from *Fusarium discolour sulphureum*, and Coons and Larmer [1929] from *Cercospora beticola* Sacc.

Zonation can be induced by fluctuation of temperature within a certain range. The data of zonation in relation to temperature are given in Table VIII.

TABLE VIII.

Relation of temperature to zonation in H. nodulosum *and* Helminthosporium C. *kept in darkness on Brown's synthetic agar.*

Temperature	Zonation		Remarks
	H. nodulosum	*Helminthosporium C.*	
10°C., 18°C. . . . Constant temperature.	*Nil*	*Nil*	....
20°C., 25°C. . . . ,,	Traces	,,	....
27·5°C., 30°C., 32·5°C. . ,,	*Nil*	,,	....
35°C., 37·5°C. . . . ,,	,,	,,	....
20°C., 30°C. . . . Alternating	X	Traces	Alternating every 24 hours.
25°C., 27·5°C. . . . ,,	XX	,,	Ditto.
25°C., 27·5°C., 30°C. . ,,	XXX	,,	Alternating every 8 and 16 hours.
32·5°C., 35°C. . . . ,,	Traces	*Nil*	Ditto.
35°C., 37·5°C. . . . ,,	*Nil*	,,	Ditto.
10°C., 18°C. . . . ,,	Traces	,,	Alternating every 48 hours.

X=slight zonation ; XX=fair zonation ; XXX=good zonation

In *H. nodulosum* at a constant temperature, zones either failed to appear or were only very faintly visible. The alternation of temperature within 20° to 32·5°C. resulted in the appearance of zones, but above or below this range zonation could not be induced. In *Helminthosporium C.* only faintly visible traces of zones were seen at alternating temperatures between 20° and 30°C.

(iv) Microscopic growth characters.

The effect of environmental conditions on various microscopic features such as sporulation, spore shape, size, septation, the colour of the conidiophores and chlamydospores is dealt with in this section.

Senescence.—In old cultures or in cultures kept above the optimum temperature. the aerial mycelium was observed to mat down close to the surface of the medium as a thin, dull glazed layer. This process has been described by Stevens [1922] for *H. sativum* as ' senescence phenomenon ' of aerial mycelium. This phenomenon was particularly marked in *Helminthosporium C.*

Sporulation.—In *H. nodulosum* the effect of exclusion of light promoted sporulation and suppression of aerial growth. When the fungus was grown in Petri dishes in darkness and after a few days the growth was exposed to light, the formation of spores was stimulated. A similar stimulating effect on sporulation could be seen by growing the fungus at low temperatures (10°C.) and, after about 10 days' growth. exposing to higher temperatures (30°C.).

Conditions of high humidity also seemed to favour sporulation. Plates with growing cultures of the fungus were held upside down over water surface and various percentages of sulphuric acid showed differences in sporulation. Conditions of high humidity from 80 to 100 per cent. favoured spore formation, while at a lower humidity of 50 per cent. its formation was entirely suppressed. Spore formation also differed on various media, on different amounts of the same medium, and on the concentration of the medium. It was profuse in darkness at 30°C. on oat-meal agar, corn-meal agar, barley-meal agar, prune-juice agar, Brown's synthetic starch agar, the sterilised green leaf of *E. coracana*, the sterilised moist straw of *E. coracana* and wheat straw, and was poor on bean-meal agar, the leaf-extract agar of *E. coracana*, and on Richards' solution agar. On Brown's synthetic starch agar spore formation was studied at various temperatures in darkness and was found best between 28°C. and 29°C., that is, at a temperature near the optimum for surface growth, while it was fairly good at temperatures ranging from 20°C. to 31°C. It was absent at 10°C. and below, also at 35°C. and above. Sporulation on Richards' solution agar of full strength was very poor but when the medium was diluted 10 to 50 times it became fairly good, while on Coons' medium it became less with gradual dilutions. Local sporulation was induced in some media by wounding the surface of the fungus colony. This gave positive result on Richards' solution agar and Brown's synthetic starch agar, while on Brown's synthetic agar and bean-meal agar it had no effect. A similar effect had been noted when an actively growing colony of the fungus was influenced by a foreign organism, *e.g.*, a bacterial colony, which exerted inhibitory influences on growth. A heavy crop of conidia was produced at the edge adjacent to the bacterial colony.

Sporulation in *Helminthosporium C.* was fair on Brown's synthetic starch agar, corn-meal agar, rice-meal agar and good on the sterilised leaf of *E. coracana*, oat-meal agar and wheat straw at 25°C. to 27·5°C. It was poor on other media. Spores were formed on the surface of the medium and later were covered with a dense aerial growth which masked their presence. They were formed most profusely between 20°C. and 27·5°C. on Brown's synthetic starch agar and over a range from 20°C. to 32·5°C. At temperatures higher than 27·5°C. the number of spores formed decreased considerably and the submerged hyphae became swollen, beaded or knotted, thick-walled, and looked like chlamydospores. Media rich in starch and higher temperatures readily gave rise to such conditions of hyphae.

Conidia.—The morphology of the conidia is greatly influenced by environmental conditions. Single spore cultures of *H. nodulosum* were grown under different environmental conditions, and it was found that there was a marked variation in the length, width, septation and colour of both conidia and conidiophores. Such variations have also been noticed by Stevens [1922], Dosdall [1923], Christensen [1922], Nisikado [1927] and Mitra [1931] in various species of Helminthosporium.

The fungus was grown on a number of media at 25°C. and the conidia were measured after ten days in every case. The details are recorded in Table IX. Fig. 1 shows the variation in the conidial lengths on various concentrations of Richards' solution agar at 25°C.

TABLE IX.

Length, width and septation of conidia of H. nodulosum *on different media at 25°C.*

Culture medium	Length in μ			Width in μ		Septation		
	Range	Mode	Mean	Range	Mean	Range	Mode	Mean
Brown's synthetic starch agar.	28·1—76·0	56	52·7	11·8—18·0	15·1	3—8	7	6·6
Normal Richards' solution agar.	22·0—64·0	40	45·2	10·0—18·0	14·6	2—8	6	5·4
N/5 Richards' solution agar.	28·0—78·0	48	50·4	11·0—16·8	14·2	3—8	6	5·6
N/10 Richards' solution agar.	30·0—78·0	56	58·7	11·0—19·2	14·5	3—9	8	6·8
N/50 Richards' solution agar.	44·0—92·0	72	62·8	12·0—19·2	15·6	5—9	8	7·2
N/100 Richards' solution agar.	36·8—88·8	72	68·1	12·0—18·0	14·4	5—8	8	7·1
Sterilised green leaf of *E. coracana*.	25·0—98·0	70	67·3	11·8—20·0	15·0	2—10	7	6·5

Length, width and septation of conidia in *H. nodulosum* varied considerably on different media.

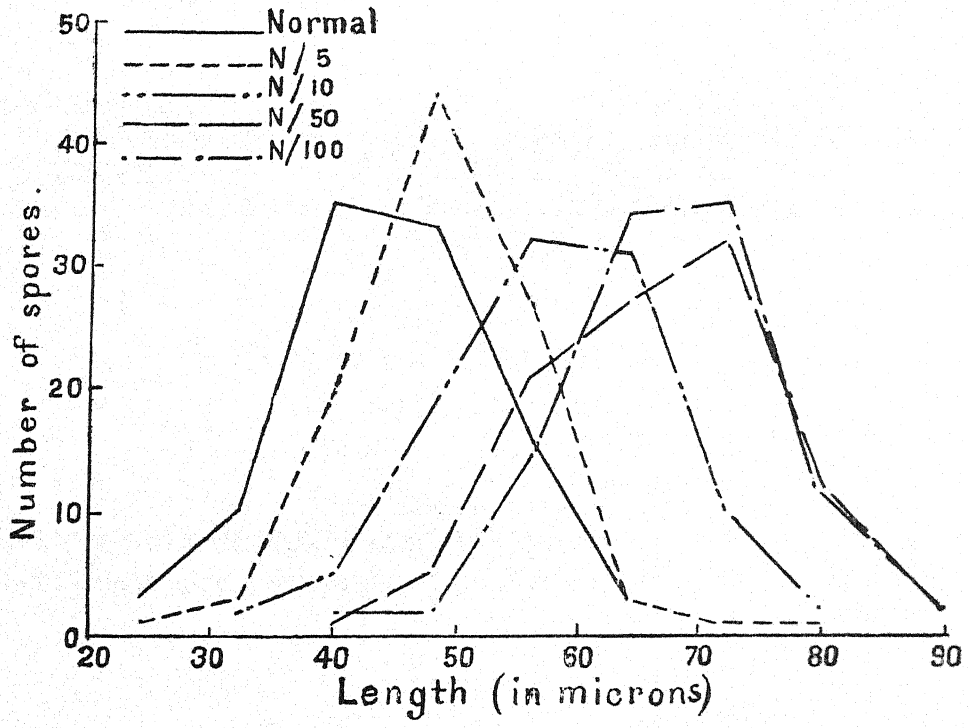


Fig. 1.—Length of conidia of *Helminthosporium nodulosum* produced on different concentrations of Richards' solution agar at 25°C.

On Richards' solution agar of full strength the average length of conidia was 45·2μ, but when this medium was diluted the length increased, *e.g.*, in Richards' solution agar *N*/5, it was 50·4μ; in *N*/10, 58μ; in *N*/50, 62·8μ; and in *N*/100, 68·1μ. Thus the average length increased by 23μ in *N*/100 solution agar. Similar variation was noticed in septation in Richards' solution agar of various dilutions. In Richards' solution agar of full strength the average number of septa was 5·4, while in a hundred dilution it was 7, that is, an increase of about two septa. The variation of the conidial septation on various dilutions of Richards' solution agar is shown in Fig. 2. These facts agree with previous observations [Mitra, 1931]. The spore width also varied with the nature of the medium but the variability was not so great as in the conidial length.

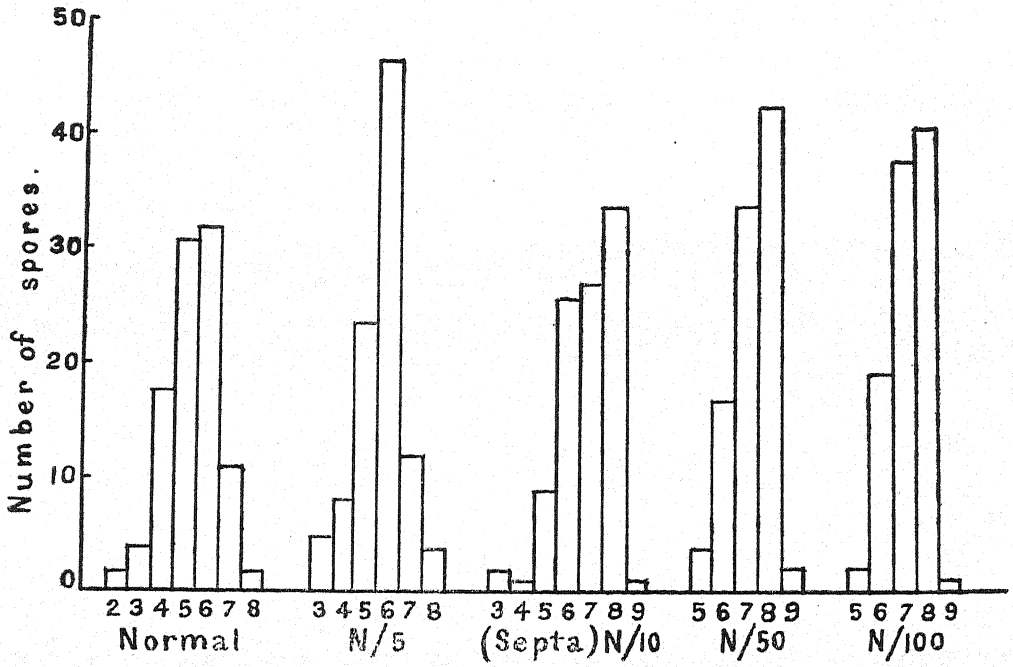


Fig. 2.—Septation of conidia of *H. nodulosum* produced on different concentrations of Richards' solution agar at 25°C.

Temperature also has a pronounced effect in modifying the size and septation of conidia. Table X shows the variation in spore size and septation at various temperatures.

TABLE X.

The effect of temperature on length, width and septation of conidia of H. nodulosum *on different media.*

Medium	Temperature in °C.	Length in μ			Width in μ		Septation		
		Range	Mode	Mean	Range	Mean	Range	Mode	Mean
Brown's synthetic starch agar.	18	40—100	72	69·3	11·0—19·0	15·3	4—8	7	6·6
Brown's synthetic starch agar.	25	28—76	56	52·7	11·8—18·0	15·1	3—8	6	5·6
Brown's synthetic starch agar.	27·5	34—70	48	50·2	12·0—20·0	15·5	3—7	5	5·4
Brown's synthetic starch agar.	32·5	22—50	32	35·6	11·0—18·0	14·2	1—5	3	3·5
Sterilised green leaf of *E. coracana.*	25	25—98	72	68·1	11·8—20·0	15·0	2—10	7	6·6
Sterilised green leaf of *E. coracana.*	31	22—78	48	48·7	10·0—18·0	14·2	1—9	6	5·5

At higher temperatures the conidia were progressively shorter. The width also became slightly reduced but was really little affected. The number of septa too decreased. The characteristic shape of the conidia was also lost. The longer conidia tended to become more or less cylindrical, while the smaller conidia were ovate or obovate.

Variation in the conidial length and septation on Brown's synthetic starch agar is shown in Figs. 3 and 4 respectively.

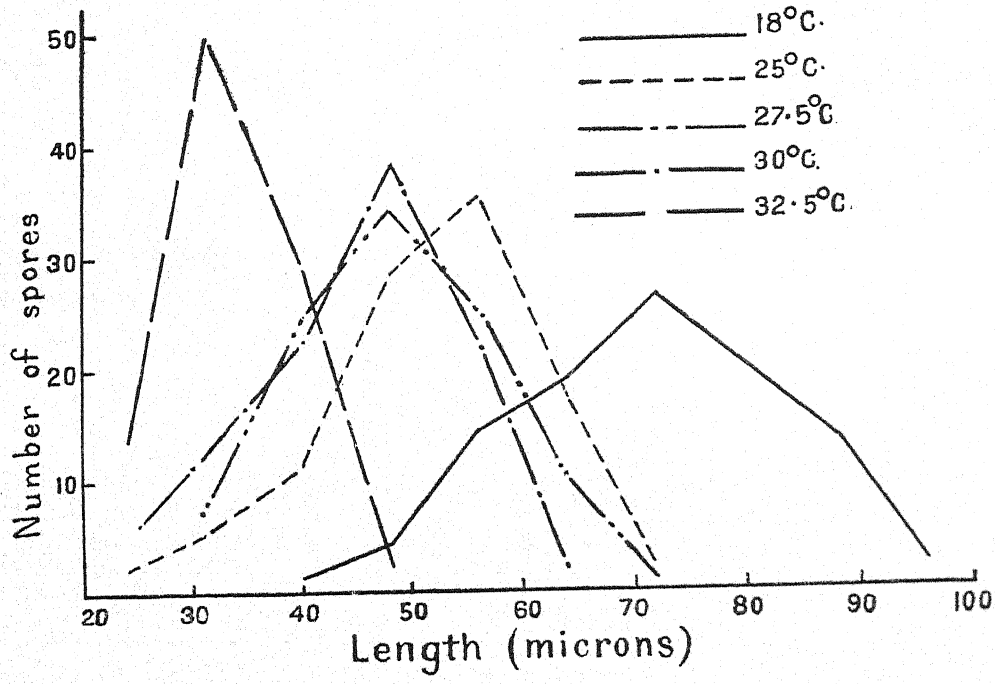


Fig. 3.—Length of the conidia of *H. nodulosum* on Brown's synthetic starch agar at different temperatures.

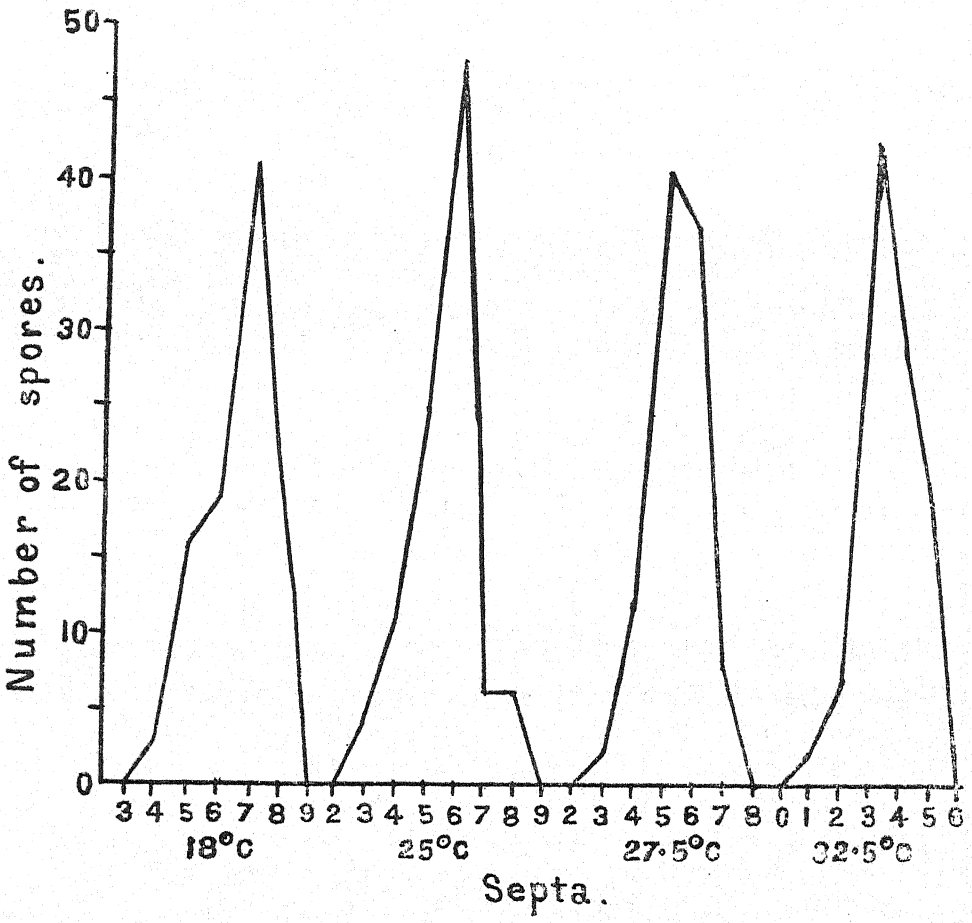


Fig. 4.—Conidial septation of *H. nodulosum* on Brown's synthetic starch agar at different temperatures.

The conidia on various meal agars, Brown's synthetic starch agar, Coons' agar, etc., at 25°C. were of a yellowish olive-green colour, while on bean-meal, Richards' solution and Brown's synthetic agars they were of dark neutral colour. In general, the tint of conidia developing at a higher temperature, *e. g.*, 32·5°C., was darker than that of those formed at lower temperatures. In some media, *e. g.*, Brown's synthetic starch agar, some spores formed at 32·5°C. showed hyaline end cells.

Conidiophore.—The shape and size of the conidiophore depends to a great extent on the number of conidia borne, and indirectly, therefore, on the nature of the media. On certain media which favour good sporulation, the conidiophores

showed many sharp bends and surface projections such as those formed on Coons' *N*/5 or *N*/10 agar and sterilised green leaf of *E. coracana* at 27·5°C. On some other media the conidiophores were very simple, almost indistinguishable from vegetative hyphae with few scars and bends. Such conidiophores were seen in culture plates on Richards' solution agar at 30°C. or on Brown's synthetic agar at 30°C.

Chlamydospores appeared in old cultures and in those grown at high temperatures. They were abundantly formed in liquid media such as Richards' solution, also on straw cultures incubated at higher temperatures. Chlamydospores developed readily in *Helminthosporium C.* on Brown's synthetic starch agar even at 24°C.

These facts show that in a single spore culture of *H. nodulosum* marked variations may be found in the morphology of conidia and conidiophores, and this probably accounts for the variations that occur under different climatic conditions.

(*v*) *Linear rate of growth.*

Temperature.—The linear rate of growth of the two isolations of *H. nodulosum* from *E. coracana* and *E. aegyptiaca* and that of *Helminthosporium C.* were studied on Brown's synthetic agar, Brown's synthetic starch agar and Richards' solution agar at various temperatures. The experiment was carried out in selected Petri dishes of uniform size into which equal amounts of the medium were poured. All the dishes were inoculated at the same time and kept at various temperatures in darkness. The experiment was run in triplicate and repeated twice. The diameters of the growing colony were measured from time to time and the data obtained are presented in Figs. 5 and 6. The curves obtained for growth rates of *H. nodulosum* isolated from *E. aegyptiaca* and *E. coracana* were similar and confirmed their identity.

H. nodulosum could grow at a temperature as low as 10°C. There was no growth at 39°C. though the fungus was not killed; by subsequently keeping the cultures at lower temperatures the fungus resumed growth though slowly. The maximum temperature, therefore, lay between 37·5 and 39°C. The optimum temperature varied with the nature of the medium, *e. g.*, in Brown's synthetic starch agar the optimum for the first few days was 30°C. and later on 27·5°C. On Richards' solution agar and Brown's synthetic agar it was 30°C. throughout.

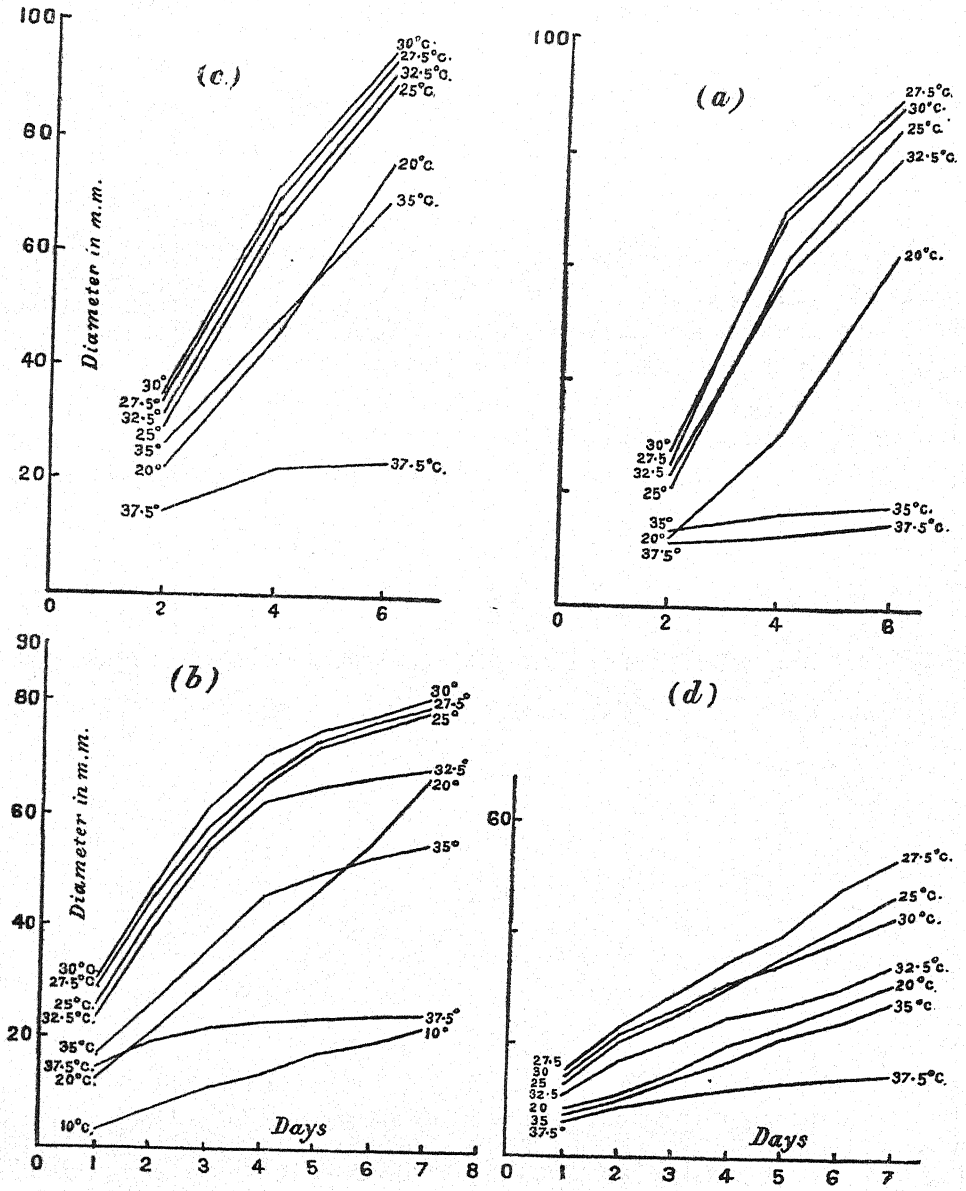


Fig. 5.—Growth rate of *H. nodulosum* (from *E. coracana*) at various temperatures on (*a*) Brown's synthetic agar, (*b*) Brown's synthetic starch agar, (*c*) Richards' solution agar and (*d*) that of *Helminthosporium C.* on Brown's synthetic agar.

From Figs. 5 and 6 it would be clear that at lower temperatures the growth continued more or less uniformly but at higher temperatures the initial rate of growth is not maintained as is indicated by the bending of the curves, *e. g.*,

curves of 32·5°C., 35°C. and 37·5°C. The bending of curves is due to staling which is most on Brown's synthetic agar and least on Richards' solution agar.

On Brown's synthetic agar there was staling at all temperatures above 20°C. On Brown's synthetic starch agar the staling was less marked at temperatures lower than 32·5°C. On Richards' solution agar and Brown's synthetic agar the staling was not so noticeable at 32·5°C.

Helminthosporium C.—Temperature relations of *Helminthosporium C.* were also determined. This is a comparatively slow growing fungus though not so staling at higher temperatures (Figs. 5 (*d*) and 6).

The optimum temperature for growth was 27·5°C. on Brown's synthetic starch agar and Brown's synthetic agar, but 30°C. on Richards' solution agar. Fig. 6 shows the comparative growth of two strains of *H. nodulosum* and *Helminthosporium C.* on Brown's synthetic starch agar after seven days.

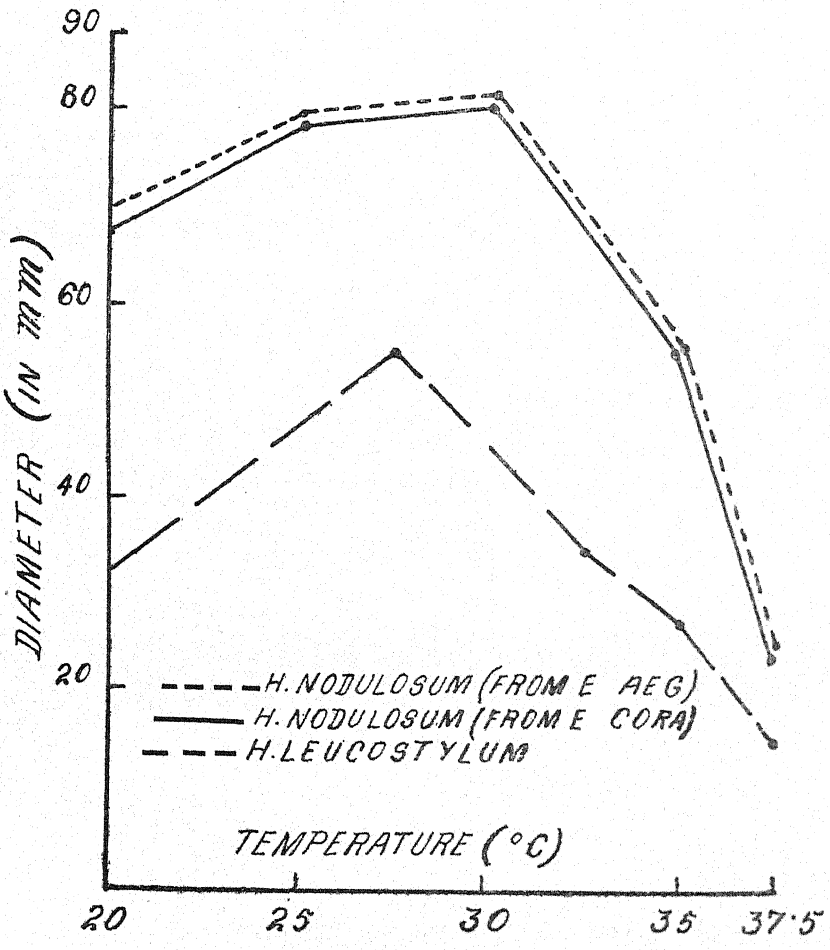


Fig. 6.—The growth of *H. nodulosum* from *E. coracana* and *E. aegyptiaca*, and of *Helminthosporium C.* at various temperatures on Brown's synthetic agar after seven days.

The results obtained in this study show that the optimum temperature differs on different media. In *H. nodulosum* there is a shift in the optimum temperature from 30°C. to 27·5°C. on Brown's synthetic starch agar. Similar results have been obtained for *H. sacchari* Butl. by Helma and Fawcett [1925] and by the latter for some other parasitic fungi [Fawcett, 1921]. The amount of staling differs with the medium used. There seems to be some correlation between higher optimum and least staling as found on Richards' solution agar.

The thermal death point of conidia of both the fungi was determined and found to be (for ten seconds' exposure) 62-63°C. for *H. nodulosum* and 59-60°C. for *Helminthosporium C.*

Depth of medium.—To determine the effects of the depths of medium on daily rate of growth of *H. nodulosum* an experiment was carried out in which plates of uniform size containing different amounts of the medium were inoculated. All these plates were kept at constant temperatures and the experiment, which was run in triplicate, was repeated several times to confirm the results.

The rate of growth was found to depend on the amount of the medium per plate. The final growth was more on thickly poured plates. Further, the thinly poured plates started staling earlier. This agrees with the observations made by Mitra [1931]. Fig. 7 shows the rate of growth on media with different depths.

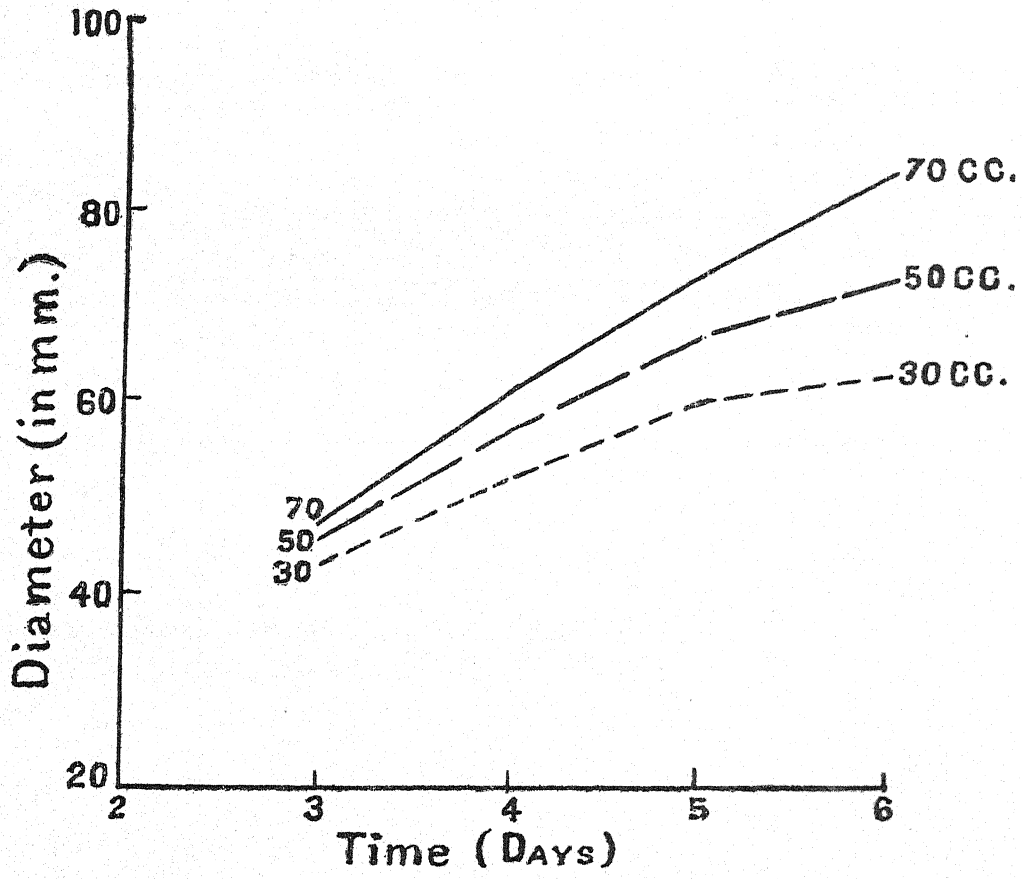


Fig. 7.—Growth rate of *H. nodulosum* on Brown's synthetic agar with different depths of the medium at 30°C.

Humidity.—Cultures were exposed to atmosphere with different degrees of humidity by using sulphuric acid of varying specific gravities prepared according to the method given by Stevens [1916]. Sterilised dishes of uniform size were used for the purpose and a known volume of the acid was put in each to fill about one-fourth of the volume. Petri dishes of uniform size in which equal amounts of the same medium had been poured and inoculated with *H. nodulosum* from *E. coracana* were fixed with gelatine solution (to which some mercuric bichloride was mixed as an antiseptic) to glass panes big enough to fit on the top of the containers. The lids of the Petri dishes were removed and the glass panes with dishes were sealed with vaseline to the containers. According to this arrangement the surface of the medium on which the fungus was growing was facing downwards

exposed to the acid solutions, exerting known vapour pressure in the manner figured and described by Paul [1929]. The linear rate of growth was measured on alternate days. The data of the measurements, which are the average of two experiments, each running in triplicate, are given in Table XI.

TABLE XI.

Linear rate of growth of H. nodulosum *in varying atmospheric humidity.*

Relative humidity	Growth in m. m.		
	3rd day	7th day	9th day
50 per cent.	22·2	55·2	65·6
70 „	19·6	58·8	74·9
78 „	14·8	61·7	76·2
92 „	11·2	69·2	78·8
100 „	8·9	54·2	65·0

The results of the experiment show that the best growth is in an atmosphere of 92 per cent. humidity though the growth is faster at lower humidities during the first three days. In an atmosphere fully saturated with water vapour the growth is slow.

V. CONCLUSION.

H. nodulosum, *H. cyonodontis*, *H. giganticum*, and *H. leucostylum* are known to occur on *E. indica* [Drechsler, 1923]. The species described as *Helminthosporium C.* does not resemble any of the above in detail but is closely allied to *H. leucostylum* in respect of small and hyaline or faintly coloured conidiophores which mostly emerge through the stomata in large groups. They also resemble in size and shape. The conidiophores of *Helminthosporium C.*, however, differ from those of *H. leucostylum* in being conspicuously slender at the basal part, gradually becoming broader at the top which is flat or anvil-shaped. The conidia of *Helminthosporium C.* resemble those of *H. leucostylum* in shape and size. The former has olive brown conidia as compared to deep olivaceous of *H. leucostylum*. The conidia of the former measure from 18-78×10-12μ, those of *H. leucostylum* measure from 15-67×11-17μ. Thus the conidia of *Helminthosporium C.* are slightly bigger than those of *H. leucostylum* and are slightly lighter in colour. The number of septa in the conidia of *Helminthosporium C.* varies from 1 to 8, on the other hand those

of *H. leucostylum* have 1-6. Thus the present fungus is closely allied to *H. leucostylum* and may be considered as a strain of the same. The slight variation in morphological characters may be due to different climatic factors which play an important part in modifying the morphology of a fungus as has been found in the present case. The authors are, therefore, inclined to include this strain in *H. leucostylum*.

VI. Summary.

H nodulosum B. et C. is widely distributed in India and causes a serious disease of *E. coracana*. The fungus was also isolated from *E. aegyptiaca*. Besides, a strain of *H. leucostylum* Drechs. was found to attack *E. coracana* but does much less damage than *H. nodulosum*.

Both *H. nodulosum* and *H. leucostylum* cause leaf spots, seedling blight and head blight. *H. nodulosum* also causes foot-rot, seed blight and root-rot. The symptoms caused by the two species are described.

Inoculation experiments show that all the parts of *E. coracana* are susceptible to the attack of *H. nodulosum* and *H. leucostylum*. Seedlings are more susceptible to the attack of *H. nodulosum*.

The optimum temperature for the infection of aerial parts by *H. nodulosum* was found to be 30°-32°C. with a range of 10°-37·5°C.

H. nodulosum infects leaves more readily from the upper surface or between the leaf and leaf-sheath. Infection takes place through the stomata, the epidermal cells, or more frequently through certain epidermal out-growths.

Cross-inoculation experiments show that both *H. nodulosum* and *H. leucostylum* have a wide host range.

The effect of temperature, humidity and plant tissues on the germination of conidia was studied and the details are described in the text.

Macroscopic growth features of *H. nodulosum* and *H. leucostylum* such as aerial mycelium, colour, zonation are affected by various environmental factors such as light and darkness, temperature, humidity of air and media, inhibitory influences.

Microscopic growth features of *H. nodulosum* such as sporulation, shape, size and septation of conidia, shape and size of conidiophores, formation of chlamydospores and secondary spores are affected by environmental conditions.

The optimum temperature for the linear rate of growth of *H. nodulosum* and *H. leucostylum* varies with the nature of the medium. *H. leucostylum* is comparatively a slow growing fungus. The linear rate of growth of *H. nodulosum* depends on a number of other factors such as the amount of medium and humidity.

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Explanation of Plates LXIV—LXVII.

Plate LXIV.

Symptoms of disease produced on *E. coracana* by *H. nodulosum* (Figs. 1—5) and *H. leucostylum* (*Helminthosporium C*). (Fig. 6).

Fig. 1. Spots in various stages of development on the leaves. A diseased inflorescence is also shown (Natural size).

Fig. 2. A diseased grainless inflorescence stalk (Natural size).

Fig. 3. Basal part of a badly affected stem (Natural size).

Fig. 4. An inflorescence with healthy and diseased spikes (Natural size).

Fig. 5. Infected leaf-sheath (*a*) with spots, (*b*) stem infected by the contact of diseased leaf-sheath (Natural size).

Fig. 6. Infected spots in various stages of development (Natural size).

Plate LXV.

H. nodulosum.

Figs. 1, 3, and 6. Conidiophore on *E. coracana* leaf, (*A*) emerging through a stomata, (*B*) emerging through an epidermic cell (×346).

Fig. 2. Conidiophore on the leaf of *E. coracana* (×500).

Figs. 4 and 5. Conidiophores from an infected inflorescence of *E. coracana* (×446).

Figs. 7 and 8. Apical portion of a branched and unbranched conidiophores from diseased inflorescence of *E. coracana* (×346).

Fig. 9. A forked conidiophore bearing conidia at an unusual position (×500).

Fig. 10. Part of a conidiophore showing the attachment of conidia (×500).

Figs. 11—15. Variation in the shape, size and septation of conidia developed on Brown's synthetic agar at 25°C. (×500).

Figs. 16—22. Variation in the shape, size and septation of conidia developed on Richards' solution agar at 32·5°C. (×500).

Figs. 23—26. Variation in shape, size and septation of conidia produced on Brown's starch agar at 30°C. (×500).

Figs. 27—31. Variation in shape, size and septation of conidia produced on Brown's starch agar at 18°C. (×500).

Figs. 32 and 33. An inoculated leaf showing the penetration of the germ-tube through the epidermis (×666).

Fig. 34. Entrance of a germ-tube through the stomata (×500).

Fig. 35. Penetration of germ-tube (shaded part discoloured as a result of the penetration of germ-tube) (×247).

Plate LXVI.

H. nodulosum.

Figs. 1—22. Conidia from the leaf and diseased inflorescence of *E. coracana* showing variation in shape, size and septation (×346).

Figs. 23—25. Conidia of *H. nodulosum* from the leaf of *E coracana* (×500).

Fig. 26. A branched conidiophore bearing conidia (×500).

Figs. 27—31. Conidia produced on green sterilised leaf of *E. coracana* at 16—18°C. (×500).

Figs. 32—39. Conidia from leaf of *E. coracana* germinating in distilled water (×500).

PLATE LXVII.

H. leucostylum (= *Helminthosporium C.*)

Figs. 1—15. Conidia from leaf of *E. coracana* (×500).

Figs. 16—24. Conidiophores from the leaf of *E. coracana* (×247).

Fig. 25. Group of conidiophores from a diseased inflorescence (×247).

Figs. 26 and 27. Conidiophores from the leaf of *E. coracana* (×500).

Fig. 28. Conidiophore emerging through a stomata (×500).

Fig. 29. Conidiophore with a small lateral branch (×500).

Fig. 30. A group of conidiophores from the diseased inflorescence of *E. coracana* (×247).

Figs. 31—34. Germination of the conidia in distilled water (×500).

STUDIES IN THE WILT DISEASE OF COTTON IN THE BOMBAY PRESIDENCY.

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Introduction.

Realising the great economic importance of the wilt disease in cotton in 1923, Dr. H. H. Mann, the then Director of Agriculture to the Government of Bombay placed a proposal before the Indian Central Cotton Committee that they should finance a thorough investigation into the wilt disease of cotton in the Bombay Presidency, to be conducted in the area which was most notorious for the disease, namely the Bombay-Karnatak. The Committee agreed to do so and the Bombay Government provided land and laboratory accommodation on the Government Experimental Farm (Station) at Dharwar. The investigation was started in September 1923. This paper presents the results of laboratory green-house and field experiments which have been carried on for nearly ten years (from 1st September 1923 to 31st March 1933). The research was a piece of team work by Mr. G. S. Kulkarni, Chief Investigator, Dr. B. B. Mundkur, Senior Assistant, Messrs. R. K. Kulkarni, and T. R. Khadilkar, Junior Assistants and Messrs. J. D. Randive, S. S. Varma and K. D. Rajalu, Post-graduate students.

COTTON RESEARCH STATION
DHARWAR,
1st April 1933.

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Chapter I.

THE WILT DISEASE OF COTTON.

Cotton is one of the important staple crops of the Bombay Presidency. It occupies, in acreage, second place among the cultivated crops and covers an area of 44 lakhs (4·4 millions) of acres. This represents about one-sixth of the total cropped area and about half of the area devoted to non-food crops. It thus occupies a foremost place among the commercial crops, and forms a main source of income to the growers. Because of its importance any factor or factors which might lower the price of the produce or affect production is bound to cause considerable economic disturbance to a large class of people such as the grower, the trader and the manufacturer. Among such factors are the diseases due to plant parasites which reduce the yield of the crop. Any diseases affecting such an important crop are, therefore, worth studying.

Distribution.

Among the various diseases to which the cotton crop is subject, the wilt disease is by far the most important. It occurs in all the cotton tracts of the Presidency except North Gujarat and Sind. Outside the Presidency, it is found in the Berar and the western parts of the Central Provinces, in the Nizam's dominions abutting on the Bijapur, Sholapur and Ahamadnagar districts, and in the northern parts (Chitaldrug) of the Mysore state. Outside India, it is known in Egypt, United States of America, Russian Turkestan, South Africa and in fact in most of the important cotton tracts of the world.

Economic importance.

In the Presidency the disease is wide-spread and the amount of damage to the crop, though considerable, varies according to localities and seasons. It is difficult, therefore, to estimate correctly the loss that is sustained. In Gujarat, some of the heavily infected tracts, especially Broach, show 20 per cent. loss of the crop. In Khandesh, oftentimes, a destruction of 30 to 40 per cent. is not uncommon. In the Karnatak, while in the heavily infected fields one can easily see a loss of up to 60 per cent., an estimate of 10 to 15 per cent. average loss is not wide off the mark. There is sufficient evidence of the increase of this trouble in recent years and the tendency of the grower to take cotton after cotton especially in the boom days, has also greatly helped its spread. On the statements of the District staff of the Bombay Agricultural Department and observations made by the investigators of the disease, an average loss of 5 per cent. may safely be put down for the whole of the wilt prevailing tracts in the Presidency. On this basis the area of the cotton crop damaged by the disease would, therefore, be 200,000 acres, taking 4,000,000 acres as the total cotton crop grown in the wilt prevailing parts (Surat and Broach districts in Gujarat, the Deccan and the Karnatak) in the Presidency. If a bale (400 lbs.) of cotton be taken as an average outturn for five acres and Rs. 100 as the price of cotton per bale, the total value of the cotton crop destroyed by the wilt disease would amount to 200,000 acres $\div$ 5 = 40,000 bales $\times$ Rs. 100 = Rs. 4,000,000. The economic importance of this disease entailing such a heavy loss is, therefore, evident.

Symptoms.

The symptoms of the cotton wilt are well known as they have been described by various investigators in great detail. A few salient points may, however, be mentioned. The disease appears about fifteen to twenty days after sowing.

The most obvious symptom of the affected plants is what is called wilting which generally proceeds in the leaves from the base upwards, the shoot finally hanging down limply. The wilted leaves may become yellow and turn brown by which time the plant is dead. Plants may succumb at any stage of their growth, from seedlings barely two weeks old to adult plants. While gradual wilting is the rule, plants which have wilted all too suddenly are not common. Plants with partial attack are of frequent occurrence. Completely attacked plants whose leaves are shed and branches killed often recover by the development of new shoots. Such plants are generally recognised by their dwarfed and bushy appearance. In fields where wilt infection is quite recent, one might find, if one carefully observes, a few wilted plants here and there soon after the seedlings come up. The attacked plants, owing to their small size, usually escape attention. They, however, serve as starting points of infection for the next crop when a few more plants might die round each infection centre, which therefore gets slightly widened. More and more plants begin to die in the succeeding crops and in a few years infected areas sufficiently large to attract attention, are formed. Such large patches are quite characteristic of the heavily infected fields. It would thus appear that a sufficiently long time is required for the disease to be recognised after its introduction.

Wilted plants do not show any discoloration externally either on the stem or root. On removing the bark, however, the woody tissues below are found blackened either in streaks or in strips. Such roots when split open show the dark streaks along the vascular strands. The streaks in advanced cases of attack could be traced down in the tap root and in most of the side roots and also higher up in the stem and branches. The microscopic study of such roots reveals the following :—In the early stage of attack there is a yellowing of the vessels and the surrounding tissue. Later on the colour may change to deep brown and occasionally to dark brown. Many of such vessels contain the fungus hyphæ which at times completely fill them. Hyphæ may be found even in vessels apparently normal, *i.e.*, without showing any discoloration. More often than not, the vessels are filled only with dark gummy masses. In bigger plants hyphæ are found not only in the darkened portions of the root and the stem but can be traced sufficiently higher up in the stem and branches and even in the fruit pedicels. A constant association of the fungs which is a species of *Fusarium* with the wilted cotton plants leads one to believe that it is the cause of the disease. The proof, of course, is obtained by inoculating healthy plants with the pure culture of the fungus and thereby producing the disease.

It is necessary to state here that the symptoms of wilting are also due to other causes. The cotton stem-borer, *Sphenoptera gossypii*, often causes a large number

of plants to wilt as also do the root-rot fungi, *Rhizoctonia bataticola* and *Sclerotium Rolfsii*. But a detailed examination of the affected plants makes it easy to distinguish the *Fusarium* wilt from the others. In the case of the borer attack there is a distinct swelling of the root, showing the tunnels caused by the insect, which are sufficiently easy to recognise; while in the *Rhizoctonia*-affected plants, the bark in the hypocotyl region is discoloured and often shredded, the stem gets weakened and breaks off readily. In the case of *Sclerotium* disease the profuse superficial matting of white mycelium of the fungus and the characteristic mustard seed-like sclerotia are quite evident.

Chapter II.

Cause of the cotton wilt disease.

Literature review.

Atkinson [1892] was the first to describe the cotton wilt disease which he did in great detail. He always found a *Fusarium* associated with the diseased plants and, considering that organism to be new, named it *Fusarium vasinfectum*. He thought that the fungus was unable to effect entry within the host unaided and his infection experiments, which were few, were carried out with this idea prominently in mind. In his laboratory tests the culture of *Fusarium vasinfectum* was introduced after the plant had been disabled by the "damping off" fungus. Both because the experiments were few and were not convincing, he left the matter in some doubt.

The wilt *Fusarium* received major attention in the extensive experiments by Smith [1899] on the disease of cow-pea, water-melon and cotton. After studying the fungus in culture and under the microscope he did his inoculation experiments. These however were not decisive. With the water-melon fungus he obtained positive results but the cow-pea *Fusarium* was rather doubtfully infective while the cotton *Fusarium* was not pathogenic at all. The perithecia of the saprophytic fungus which is a Pyrenomycete and which usually occurs on the outer surface on wilted plants led him into supposing that it was the perfect stage of Atkinson's *Fusarium* which he renamed as *Neocosmospora vasinfecta*, thus establishing a new genus. He was never able to obtain positive results in the infection tests with this fungus. The few positive results mentioned above were isolations from tissue obtained from the inside of diseased plants.

In India attention was first drawn to this disease by Evans [1908]. He found it doing much damage to the crop on the Nagpur Experimental Farm. Both

Rhizoctonia root-rot and *Fusarium* wilt are present on this Farm. In writing his description Evans evidently considered both these diseases to be manifestations of the same fungus. The material on identification proved to be true *Fusarium* wilt, for the disease was reported to be due to *N. vasinfecta*, by which name the fungus was then known.

Butler's [1910, 1926] observations on the disease are recorded in two separate reports. In 1910 he used cotton plants as the host to test the parasitism of *N. vasinfecta* and disapproved Smith's belief that the fungus was the perfect stage of *F. vasinfectum*. Detailed experiments to test the parasitism of *Fusarium* done in 1912-13 are given in the later publication.

For these latter experiments diseased material was obtained from Berar. Sterilised soil was used. In four pots single inoculations and in four others double inoculations were made. Four pots were checks. The results were not however very uniform. In one pot of the latter (double inoculation) batch and two of the former negative results were recorded. In the rest wilt prevailed though all the plants did not succumb. Butler states that the infected plants die in a manner characterstic of *Fusarium* wilts and he attributed the capricious results to some factor which he thought was not associated with the soil. That Butler's prediction was correct will be clear later in this paper where it is shown that soil temperature is a deciding factor in producing wilt. Butler did not consider that his few experiments had solved the problem of cotton wilt in Central and Western India.

The experiment reported by Ajrekar and Bal [1921] did not contribute anything decisive so far as the parasitism of the fungus was concerned. Out of thirty-two pots that were infected with culture of the fungus and contained about one hundred and sixty plants, only six plants in two pots showed symptoms of the disease. Their conclusions that the Indian *Fusarium* was less virulent or that its virulence had been affected by continued growth on artificial media is therefore quite natural. In a later communication Ajrekar [1926] narrates details of experiments to prove the parasitism of the *Fusarium*.

The pathogenicity of the American strains of *F. vasinfectum* was established beyond any possibility of doubt by Neal [1927] who found that if the experiments are conducted with proper precautions the fungus is not only pathogenic but quite virulent.

Fahmy [1928] reports successful experiments with the Egyptian strains of *F. vasinfectum*. Diseased plants for isolating the fungus were obtained from thirteen localities and two types of cotton were included in the experiments. All the thirteen isolants from the thirteen districts were uniformly pathogenic to the

type Ashamouni. In the case of the type Sakel, the *Fusarium* from the six isolations was not pathogenic, the other seven being pathogenic in different degrees. The Egyptian strain was mildly parasitic to Indian cottons and also American (Sea Island) cottons but the Indian strain was non-pathogenic both to Egyptian and American cottons, in Egyptian soils and under Egyptian conditions.

Kulkarni [1928] tried to see what may have affected the parasitism of *F. vasinfectum* in Butler's [1926] and Ajrekar and Bal's [1921] experiments. He observed that pouring the contents of a culture mixed in water on the surface of the soil was ineffective. Better success was recorded if cultures of the fungus were intimately mixed with the whole of the sterilised soil in the pots. If sowing in these pots was delayed by a month, good results were invariably obtained. In none of his checks did he note any deaths even though the soil for all the pots came from the same source and received identical treatment, excepting that it was not infected with the fungus.

Fikry [1932] isolated three *Fusaria* from wilted cotton plants from Egypt, *F. orthoceras* App. et Wr., *F. vasinfectum* Atk., and *F. angustum* Sherb., all of which were pathogenic to cotton and caused typical wilt.

The experiment done at Dharwar after the publication entitled " Studies in the Wilt Disease of Cotton in the Bombay-Karnatak "* were to reisolate the fungus and test its parasitism over again. Studies on the morphology of the fungus, its cultural characters and systematic position together with a short discussion on the aluminium toxicity theory enunciated by Dastur [1924, 1929] are also reported below.

Isolation studies.

In freshly wilted plants the mycelium of the fungus is confined to the vascular tissues only. It is possible, therefore, to subject the outer surface to a rather drastic treatment so as to be sure that the fungus obtained in culture is from the interior only. The tap root is therefore first washed carefully in running water ; washing by mercuric perchloride (1 : 1000) solution and alcohol follows. The material is then cut into small pieces with a flamed scissors and the pieces are placed in a sterilised Petri dish. With sterilised needles, forceps, and a pair of scissors the material may now be peeled, further flamed if necessary, and placed on potato dextrose agar in a Petri dish.

The mycelium can be seen the very next day with a hand lens in the agar. In three or four days it becomes visible to the unaided eye.

* The data have been published in full under the above heading by the author in the *Memoirs of the Department of Agriculture in India, Botanical Series*, Vol. XVII, No. 2, October 1928.

Single-spore cultures were obtained from such isolations by Keitt [1915] method and in all the experiments that are to be reported hereafter in this publication, the cultures used were those described here and their parasitism had been tested as described below.

For the purpose of soil inoculation the fungus was grown on Richards' liquid medium. Two litres of this medium was placed in a five-litre Pasteur flask and sterilised in the autoclave at fifteen pounds pressure for about twenty minutes. After the flasks were inoculated with the fungus culture they were placed on a laboratory bench. The fungus was allowed to grow for nearly a month by which time a thick mat of mycelium had formed on the top, with plenty of mycelium within the liquid.

The soil for these and all other experiments reported herein was from wilt-sick fields. It was sterilised for an hour at thirty pounds pressure. The soil from one sterilisation was enough for three pots which were about nine inches in diameter, twelve inches deep, and cylindrical in shape. The soil after sterilisation was allowed to cool rapidly on an iron sheet washed with a strong copper sulphate solution. The contents of one Pasteur flask were then poured on the soil mass and the fungus and soil were intimately mixed. The mixture was then potted. Sowing was done about a month later, by which time the fungus had completely infested the soil.

The pots were all sown with a susceptible type of cotton, the seed of which was delinted with concentrated sulphuric acid and further disinfected with mercuric perchloride solution.

There were thirty pots with ten seedlings each. All the seedlings in the wilt-infested pots manifested symptoms of the disease in about fifteen to twenty days and were all dead by the end of the month.

Each of the dead plants was microscopically examined and the fungus was found in each one of them.

A certain number of isolations was made and the fungus obtained compared very well with the standard culture with which the work was started.

These isolations were tested for their parasitism and they reproduced the disease.

There were no deaths in the check pots.

Included in these tests were a few other pots as well and the observations made thereon may now be set forth.

In addition to thirty pots with infested soil and ten check pots, there were ten pots with unsterilised soil from wilt-sick fields. Each pot had ten plants. Out of the total of 100 plants seventy plants died.

Another set of fifty pots was filled with a reddish coloured soil from the bottom of a tank in Dharwar city. Thirty of these were filled with sterilised and infected soil, ten with only sterilised soil, and ten with the same reddish soil but unsterilised. Fifty per cent. of the plants in fungus-infested pots died showing typical wilt. Plants in sterilised and unsterilised checks remained healthy.

In another set of fifty pots black cotton soil from a field, where wilt had never been seen before, was filled in the same manner as above. Ten pots contained unsterilised soil. Another ten contained unsterilised but fungus-infested soil. A third ten were filled with sterilised soil. Twenty more were filled with sterilised and fungus-infested soil. About eighty per cent. deaths were recorded in both unsterilised infested and sterilised infested soils. No deaths occurred in the pots wherein the fungus had not been introduced.

It should be emphasized that wilted plants were always carefully examined both macroscopically and microscopically and they were declared to have wilted only when the fungus was seen in the tissues and later isolated on nutrient agar.

The experiments described on the previous page are recorded in Table I.

TABLE I.

Results of experiments to test the parasitism of Fusarium *isolated from wilted cotton plants.*

Kind of soil	Treatment	No. of pots	Total No. of plants	Deaths
Wilt-sick soil . . .	Unsterilised	10	100	100. All showed fungus.
	Sterilised	10	100	*Nil.*
	Sterilised infected . . .	30	300	300. All showed fungus.
Red soil . . .	Unsterilised	10	100	*Nil.*
	Sterilised	10	100	*Nil.*
	Sterilised infected . . .	30	300	150. All showed fungus.
Wilt-free black cotton soil	Unsterilised	10	100	*Nil.*
	Unsterilised infected . .	10	100	70. All showed fungus.
	Sterilised	10	100	*Nil.*
	Sterilised infected . . .	20	200	160. Fungus seen in all cases.

Cultural studies.

The *Fusarium* isolated from cotton plants grows well on most of the common laboratory media. On potato slabs in Roux tubes a very profuse white woolly

growth of the mycelium takes place. Within four days the conidia begin to appear and as the culture ages chlamydospores are formed and begin to predominate.

On sterilised carrot pieces growth is rather sparse and consists of thick white hyphae having a larger proportion of microconidia than macroconidia. In old cultures the hyphae are practically cut up into chlamydospores.

Very little growth occurs on cotton leaf mulch to begin with but later it considerably increases. The mycelium is slightly pinkish and is usually confined to the surface. A profuse formation of chlamydospores with few microconidia and macroconidia is a particular feature on this medium.

On tomato stems the fungus forms a profuse growth of white woolly mycelium with a large number of chlamydospores and microconidia. Macroconidia are none or few.

On rice grains steamed in test tubes the fungus grows the best. The aerial growth to begin with is white but later the colour changes to light pink, then deep pink and sometimes to purple. Unicellular conidia are usually formed. Strands can be seen on the sides of the tubes. Copious formation of chlamydospores with plenty of microconidia is a feature of older stages in cultures but macroconidia are rather few.

On rice with a few drops of hydrochloric acid pink colour appears rather quickly. It may then pass through violet and then purple stages. On rice made alkaline by adding a few drops of saturated sodium carbonate, pink colour is not developed. Violet colour can however be noted after the tubes have aged a little. The growth of mycelium is not profuse as on acidified rice and the spores are mostly microconidia.

The fungus was also grown on a few liquid media. Butler's [1910] standard solution was used as the basic medium. Its composition is as follows:—

Ammonium nitrate	10·00 grms.
Potassium biphosphate	5·00 „
Magnesium sulphate	2·50 „

After adding the requisite carbon source the solution was made up to a litre.

The experiments were conducted in Erlenmeyer flasks of one hundred c. c. capacity. Each flask held twenty c. c. of the respective medium. The flasks were all properly sterilised in the pressure cooker.

On Butler's medium with three per cent. glucose a profuse growth of mycelium of the pinkish colour within ten days was observed. Not much growth formed within the solution itself. The hyphae formed strand-like structures sometimes giving out short lateral branches. In many of the hyphae a regular row of oil globules was noticed. Only a few microconidia and chlamydospores were seen. In about

twenty days the surface growth had become dense, the pink colour had faded, and the colour was creamy white.

With three per cent. saccharose the growth characteristics were same as before excepting that after a month's time, a slight orange colour appeared.

In Butler's medium with three per cent. maltose surface growth was similar as in the above two cases but there seemed to be a discouragement to spore formation. A faint orange colour became noticeable in the old flasks.

In Butler's medium with five per cent. glycerine there was no aerial growth. But dense submerged growth white in colour to begin with, but later becoming deep pink, became noticeable in about fifteen days. There were also a few macroconidia and chlamydospore which appeared rather profusely.

On peptone, one per cent. of which was added to Butler's medium, dense growth was formed which also reached the surface. It was not dense however on the surface. It developed a creamy white colour but no microconidia or chlamydospores were formed.

On asparagin of which one per cent. was added to the standard medium, more submerged but less surface growth was formed. The colour was at first pink but later it became creamy white.

When 0·5 per cent. absolute alcohol was added to Butler's medium, the growth was again confined to the liquid, very little having formed on the surface. A few chlamydospores were formed but no micro- or macroconidia.

No difficulty has, therefore, been found in cultivating this fungus on artificial media. In addition to the media mentioned above the fungus has been grown in the course of the past eight years on such media as hard potato agar, potato glucose agar, potato starch agar, Brown's synthetic agar, oat agar and corn-meal agar. In all these media the fungus grew profusely colouring the substratum pink or various shades of violet. On some the growth was woolly and on others its consistency and texture was coriaceous with a creamy colour. One or two-celled microconidia predominated and macroconidia were fewer. In older cultures a large number of chlamydospores was in evidence. Abortive attempts to form sclerotia-like bodies were sometimes made but regular sclerotia were not observed.

The fungus has been grown in a large number of various liquid culture media in addition to those mentioned above. Coons' medium, Raulin's medium, Duggar's medium, Richards' medium, Dox's medium, Wichards' E medium as modified by Wolpert have all been tried in some connection or other and the fungus has made satisfactory growth. The fungus seems to prefer glucose and saccharose most for its carbon sources, the growth on other carbon sources being not very profuse.

When carbon is supplied in the form of alcohol (absolute alcohol and glycerine) or in the form of organic acids (citric acid and lactic acid) it can be utilised also.

Fungi show a marked specificity in their affinity to their carbon and nitrogen sources. Their food relation may constitute an important factor determining their parasitism on the one hand and the resistance or immunity of their respective hosts on the other. The obligate parasites are fastidious regarding their food requirements but facultative parasites are less so, yet the stereochemical differences in various culture media may affect their growth. When the question of the predisposition of a fungus to be parasitic or of the host to be resistant or immune is considered, the results of such a study may have important bearing. An endeavour was therefore made to study the influence of various carbon sources and nitrogen sources on the growth of this *Fusarium*.

Carbon sources.—In order to determine the influence of various carbon compounds the effect first of the following sugars was tried : Glucose, fructose, galactose saccharose, maltose, lactose, soluble starch, inulin and arabinose. Two per cent. of these sugars were added to the following standard solution.

Ammonium nitrate	5·00 grms.
Postassium biphosphate	2·50 „
Magnesium sulphate	1·25 „
Iron salt	A trace.

Made up to 500 c.c. so as to have in double strength.

Fifty c.c. of the above solution were placed in a 100-c.c. measuring flask, two grms. of the necessary carbohydrate were added, and made up to 100 c.c. In Erlenmeyer flasks of 100-c.c. capacity, thirty c.c. of the solution were placed, the flasks were plugged, and sterilised in the autoclave. There were three flasks for each sugar.

After sterilisation had been effected, the flasks were inoculated with small pieces of agar on which the *Fusarium* had been growing. These pieces were as uniform as possible. The fungus was incubated in an incubator adjusted to above 27°C. for fifteen days.

Best growth seemed to be forming in those flasks where inulin was introduced and the next best in the flasks with fructose supplied as the carbon source. In lactose growth was very scanty.

On the sixteenth day further growth of the fungus was terminated by adding a few c. c. of formaldehyde. The dry weight of the mycelium that had formed was determined by filtering the contents of the flasks through weighed Gooch's crucibles and drying these latter to constant weight in a hot water oven. The figures given in Table II form the total quantity of the growth in the three flasks.

TABLE II.

Growth of the Fusarium *from wilted cotton plants in culture solutions containing different carbohydrates.*

Carbohydrate	Total weight of growth in mg.	REMARKS
1. Glucose	224·0	Plenty of surface growth.
2. Fructose	303·8	As above.
3. Galactose	256·2	Both surface and submerged growth.
4. Saccharose	326·5	Plenty of surface growth.
5. Maltose	290·3	More submerged growth.
6. Lactose	11·6	Little submerged growth.
7. Soluble starch	166·8	Plenty of surface growth.
8. Inulin	320·3	Best growth of the lot.
9. Arabinose	314·9	Growth as in fructose.

Even though the growth formed in inulin flasks seemed to be the best and that in fructose flasks the next best, yet when quantitative measurements became available saccharose recorded the best growth. Only lactose did not encourage the growth of the fungus.

Two other carbohydrates which belong to what are known as higher alcohol series were also included in the tests. Two per cent. of these were added to the basic solution and the growth rates are recorded in Table III. The growth formed on saccharose is also recorded for the purpose of comparison.

TABLE III.

Growth of Fusarium *from wilted plants in culture solutions containing higher alcohols.*

Carbon source	Total weight of growth in mg.	REMARKS
Glycerine	273·0	Not much surface growth (Pinkish).
Mannite	300·8	Both surface and submerged.
Check (sucrose)	326·5	....

The *Fusarium* was able to get its carbon supply from these higher alcohols. In mannite it formed good growth.

For studying the nitrogen demands of the *Fusarium* it was grown on the same standard solution as before, excepting that ammonium nitrate was withdrawn. Instead, one per cent. ammonium nitrate was added to one set of flasks, and the other nitrogen compounds taken were such that they held nitrogen equivalent to the former compound, *i.e.*, 1·3 per cent. of ammonium chloride, 2·13 per cent. of ammonium nitrate, 1·73 per cent. of sodium nitrate and 2·53 per cent. of postassium nitrate. The other details of the experiments were as before.

The nitrogen compounds added, it will be noticed, are all inorganic compounds. However the following three compounds with nitrogen in the organic form were also included in the study: Leucine (1·99 per cent.), asparagine (one per cent.) and peptone (2·00 per cent.). The nitrogen in the leucine is equivalent to that in one per cent. asparagine.

TABLE IV.

Growth of Fusarium *from wilted plants in culture solutions containing various nitrogen compounds.*

Nitrogen source	Total weight in mg.	Remarks
Ammonium nitrate	103·1	Good growth, aerial.
Ammonium chloride . . .	66·5	Scanty submerged growth.
Sodium nitrate	211·1	Good aerial growth.
Do.	*Nil.*	....
Potassium nitrate	206·6	Good woolly growth.
Leucine	213·4	More submerged than aerial.
Asparagine	257·3	Good woolly growth.
Peptone	346·0	Both submerged and aerial growths.

Ammonium with the chloride radicle and sodium nitrate did not favour growth of the fungus while peptone led as the best nitrogen compound. All the others supported a good growth.

The rather omnivorous nature of the cotton wilt *Fusarium* was revealed by these studies. No nutritional peculiarities of the fungus which would have been

useful in explaining the preference by this fungus of certain types of cotton to others, were discovered.

Systematic position.

The systematic position of *Fusaria* is difficult of determination. Several attempts have been made to standardise the methods adopted in the taxonomic study of these fungi. Identification can, however, still be done by a few specialists. Cultures of *Fusaria* isolated from wilted cotton plants were submitted in December 1917 to the judgment of Dr. H. Wollenweber of Berlin. The following are the extracts from a communication from him :—

"I consider the fungus same as the *Fusarium vasinfuctum* Atk. var. *inodoretum* Wr. described in *Phytopathology* 3 (1913), 29, and described in monograph on *Fusaria* of *Gossypium harbaceum* Carolina No. 377, *Ann. Mycol.* 24 (1917)."

A comparative study of four isolations, one from Dharwar, another from American material kindly sent by Dr. V. H. Young of Arkansas Experiment Station, a third described by Jiwan Sing [1927] as strain A and kindly sent by Mr. J. F. Dastur, Mycologist to the Government of C. P. and Berar and a fourth isolated from Broach (Gujarat) wilted plants, was also made at Dharwar.

It has already been stated that the Dharwar isolant forms more microconidia than macroconidia. Proportions for macro- and microconidia formed by these different cotton *Fusaria* were first determined.

The fungi were grown on potato dextrose agar, incubated at an even temperature (35°C.) and counts were made after the growth had taken place for five weeks. For each strain five slides were examined and on each slide four fields were observed and the relative number of microconidia and macroconidia was counted. In order to minimise space, in Table V are recorded only the relative total of microconidia and macroconidia.

TABLE V.

Proportion of microconidia and macroconidia in different cotton Fusaria.

American *Fusarium*		Nagpur strain A		Dharwar *Fusarium*		Broach *Fusarium*	
Micro.	Macro.	Micro.	Macro.	Micro.	Macro.	Micro.	Macro.
1145	48	1555	13	59	4	346	9
23·8 : 1		119·6 : 1		14·8 : 1		38·4 : 1	

The Dharwar and American strains formed on the whole more conidia of both kinds and the totals, therefore, are small. On the whole the number of macroconidia is not large in any of the four cases.

When it became evident that there is some difference in macroconidial formation, an endeavour was made to see if there was a difference in spore dimensions. One hundred macroconidia and the same number of microconidia of each strain were therefore measured. The results are recorded in Tables VI and VII.

TABLE VI.

Biometrical constants of the length of macroconidia of four strains of Fusarium vasinfectum.

Strain	Mean	Standard error	Coefficient of variability
American	23·35±·38	5·76±·28	24·6±1·2
Dharwar	16·21±·22	3·15±·15	19·4±0·93
Broach	20·35±·32	4·77±·23	23·4±1·12
Central Provinces A strain	18·55±·38	5·72±·27	30·8±1·48

TABLE VII.

Biometrical constants of breadth of macroconidia of four strains of Fusarium vasinfectum.

Strain	Mean	Standard error	Coefficient of variability
American	3·61±0·034	0·507±·024	14·04±·67
Dharwar	3·43±0·033	0·495±·024	14·4±·69
Broach	3·36±0·014	0·447±·021	13·3±·64
Central Provinces	3·43±0·007	0·105±	..

The following conclusions can be drawn from the data presented in the above tables: The American macroconidia are the longest, the next come the Broach, then the Central Provinces A strain, and then the Dharwar strain. In respect to variability, the Central Provinces strain shows the most, and then the American. The Broach and Dharwar strains follow. In respect of breadth, the American strain is again the broadest, then come the Dharwar and Central Provinces and

Broach in order. The Central Provinces strain shows great variability with regard to breadth also.

Whether the differences in the mean length and mean breadth of the strains are or are not significant was also determined by using the Bessel's equation.

The results are recorded in Table VIII.

TABLE VIII.

Biometrical analysis of spore measurement data of four strains of Fusarium vasinfectum.

Fusarium strains	Length		Breadth	
	Mean difference	Odds	Mean difference	Odds
American *vs.* Dharwar .	7·15±·137	1350:1	0·18±0·047	95: 1
American *vs.* Broach . .	3·00±·157	4:1	0·25±·137	434700:1
American *vs.* C. P . .	4·80±·170	16:1	0·18±·047	96:1
Dharwar *vs.* Broach . .	4·15±·121	44:1	0·07±·036	4:1
Dharwar *vs.* C. P. . .	2·35±·137	3:1	*Nil*	*Nil.*
Broach *vs.* C. P. . . .	1·80±·157	1:1	0·07±·016	*Nil.*

The analysis has shown that the American strain in respect of length differs significantly from the Dharwar strain but that the Broach and the Central Provinces (C. P.) agree with it. In respect of breadth, the American strain is significantly broader than the Indian strains studied. Both with respect to length and breadth the Indian strains do not significantly differ among themselves.

Preliminary trials showed that the ability to produce colour on steamed rice grains could be used as a character for distinguishing various strains. If a strain produced deep purple or violet colour, transplants from that culture showed this same tendency. If a strain produced a bright red colour, this was reproduced in the transplants. It did not form the purple colour. Some strains produced only a creamy white colour and they never produced any pink or violet colour.

The differences in the spore measurements of the strains studied and their differential ability to produce various colours on rice indicated that there may be physiological specialisation in this species. The word indicated has been cautiously

used. The number of spores measured was not large and the colour inheritance studies were not exhaustive. No precautions were also taken to see if there was any saltation or dissociation. Extensive pathogenicity tests have to be conducted also.

Fahmy's [1928] inability to make the Indian strains sent to him pathogenic on both Egyptian and American cottons, the rather mild manner in which the Egyptian fungus attacks either Indian or American cottons, which results have been confirmed at Dharwar, indicate that there is parasitic specialisation in the *Fusarium vasinfectum* attacking cotton. If then there is such specialisation, it becomes necessary to consider whether the different strains are varieties of *F. vasinfectum* or merely physiologic forms.

The variety *Fusarium vasinfectum* (Atk.) var. *inodoratum* Wr. was separated by Wollenweber [1913] from the parent species because of its inability to give a strong lilac odour on steamed rice. But this is hardly a good distinguishing character especially when it is taken into consideration that even though Wollenweber failed to detect that odour in the particular isolant sent to him for identification, the odour has been noted being emitted by several other strains studied at Dharwar, when grown on rice. It may be better, therefore, to call the Dharwar *Fusarium*, *Fusarium vasinfectum*.

Fahmy [1928] reports an extensive study comparing the Egyptian, Indian and American strains. The variations noted by him, however, are not such as to justify the separation of the Egyptian strain into a separate variety, *F. vasinfectum* (Atk.) *Egyptiacum*, as done by him. The differences are such as would be shown by the same species from different geographic locations. Indeed the differences shown by the Broach and Central Provinces strains are such that they can get varietal rank if Fahmy's criteria are adopted. It is safer to consider Fahmy's *Fusarium* therefore as a mere physiologic form than a separate variety of *F. vasinfectum*.

Butler [1926] has recently given some cogent reasons to doubt the specific rank given to several hemisaprophytes, that is, species mainly parasitic but capable of a saprophytic life. With regard to Fahmy's comparative study of Indian, Egyptian and American strains he states that the Indian strain deserves specific rank, if specialisation of parasitism is the guide. With regard to sesamum (*Sesamum indicum* L.) wilt *Fusarium* and *Fusarium cubense*, Butler mentions that comparative studies failed to show any morphological differences, and as regards *F. udum* and *F. vasinfectum*, he reports that the earlier cultural differences have broken down. Recently Butler and Bisby [1931] have made *F. udum* a synonym of *F. vasinfectum* and have designated the latter as the cause of the wilt in pigeon-pea (*Cajanus indicus*), cotton and sesamum. For these and other reasons already stated above,

it is suggested that Wollenweber's variety *F. vasinfectum* (Atk.) *inodoratum* and Fahmy's variety *F. vasinfectum* (Atk.) *Egyptiacum* may not after all, be valid.

The question then arises whether there will not be considerable confusion such as existed in the case of *Puccinia graminis* which causes the stem rust of several cereals and grasses before the trinomial system was adopted in their case, if all the vascular *Fusaria* of the section *elegans* are referred to the species *Fusarium vasinfectum*. At present the *Fusaria* causing the wilt of cotton, pigeon-pea, sesamum and sann hemp [Hansford, 1925] have all been referred to this species.

Butler in the paper already referred to considers that there may not be much even in host specialisation and provided proper conditions are available, such host specialisation may break down. While this must be studied in the future the present work leads one to believe that without creating any new species it would be a sound plan to adopt the system of trinomial nomenclature so that while handling a culture of *F. vasinfectum* an investigator knows which particular race he is studying. With this view it is proposed to all these various strains of *Fusarium vasinfectum* as follows :—

1. The one from cotton to be known as *Fusarium vasinfectum gossypii*.
2. The one from piegon pea to be known as *F. vasinfectum cajani*.
3. The one from sesamum to be known as *F. vasinfectum sesami*.
4. The one from sann hemp to be known as *F. vasinfectum crotolariae*.

It is further suggested that within the variety *F. vasinfectum* the forms already known, the Arkansas's strain (from V. H. Young), the Dharwar strain and the Egyptian strain described by Fahmy, may be designated as forma 1, forma 2 and forma 3. Should the Broach strain and the Nagpur strain (Dastur's A strain) be found to be distinct after an exhaustive study, they may be forma 4 and forma 5 respectively.

Aluminium toxicity theory.

Within the past few years Dastur [1924] has introduced a new concept with regard to the cotton wilt disease occurring in the Central Provinces in general and on the Nagpur Experimental Farm in particular. He suggests that cotton plants absorb aluminium and iron compounds and that this may have some correlation with this disease and that the *Fusarium* isolated from wilted cotton plants may have been a contributory factor in producing the disease.

The facts which led Dastur to doubt the parasitism of *Fusarium vasinfectum* and enunciate a new hypothesis are the following :—

1. The symptoms manifested by cotton plants wilting at Nagpur are not the same as those described by other investigators.
2. *Fusarium* is not found in all the wilted plant.

3. Hundreds of pot experiments done by him have failed to establish the parasitism of isolated *Fusaria*.
4. Wilted plants show certain microchemical reactions usually not shown by healthy plants which reactions are characteristic of aluminium and iron compounds.
5. *Fusaria* do occur in soils where no wilt has been ever recorded.

Dastur's view has been criticised by Ajrekar [1926] and Neal [1927] from the mycological and by Bal [1926] from chemical point of view. But it is not proposed to go into the controversy here. Attention should be invited, however, to the following points which come out from the present work and have a bearing on the question :—

1. *Fusarium* has been invariably isolated at Dharwar from wilted specimens from various localities including Akola and Nagpur and this has been proved to be parasitic on cotton plants.
2. *Fusarium*-wilted cotton plants have been noticed by one of the Dharwar workers (G. S. Kulkarni) both at Akola and Nagpur. There is no reason to suspect that the symptoms exhibited there are not the same as those shown by the wilted plants at Dharwar, Egypt or the United States of America of which the author has personal experience.
3. As will be seen from Table I above the disease did not appear in unsterilised soil from wilt-free fields. When the same soil was infected by the fungus, seventy per cent. deaths were recorded. In sterilised soils there is no disease but when the same sterilised soil is infected by the fungus, deaths ensue.

Chapter III.

Dissemination of the cotton wilt disease through seed.

Introduction.

The cotton wilt *Fusarium* belongs to the class of half-saprophytes, *i.e.* fungi which commonly occur as saprophytes but become parasitic under certain conditions, on encountering suitable hosts. It is one of the soil dwellers that are capable of saprophytic life for long periods. Thus it has been known to survive for seven years in a field which was not sown with a susceptible cotton during the period. The disease is, in this way, a soil-borne malady. But general observations have shown that its spread from field to field is rather slow. Apparently the diseased

plants are a source of danger since the fungus is found in every part of affected plants and the large amount of debris of such attacked plants—leaves, branches and roots—when blown by the wind may carry infection to the neighbouring fields. But such a source of trouble does not seem to be an important factor as the fields adjoining the infected ones often remain free from wilt. Probably the fungus distributed through such parts of the affected plants is unable to live long exposed as these parts are to the dry weather and the sun. It has been found that during the hot months of March, April and May the surface temperature of the soil reaches 60°C. and remains at this figure for more than a couple of hours during the middle of the day. As will be noted in the next chapter (page 1013) a half-hour's exposure to this temperature completely kills the fungus. Artificial infection of surface soil with a fungus culture during the hot season has also been a complete failure. The chances of the fungus spores being blown over distances by the wind are very few as the fungus rarely fructifies on the aerial parts of the affected plants except during periods of prolonged rainy and cloudy weather and such times are rare.

The other means by which local dissemination may take place are (1) the feet of the cattle and implements which carry soil attached to them and (2) water by which soil washings take place in rains.

But to account for wilt in new areas widely separated from the infected ones, the possibility of seed being a source of infection was suggested. This suspicion gained a strong support from the complaint raised by the cotton growers that the disease is spread through the seed. It may be mentioned that the Bombay Agricultural Department was carrying on a scheme of seed distribution of improved variety of cotton for a number of years. The variety was Dharwar No. I but it happened to be susceptible to wilt. Soon after the seed distribution propaganda was started in 1917, reports regarding the outbreaks of wilt in different parts of districts began to reach the department. Later on the disease spread to such an alarming extent that the Department had to stop the distribution of Dharwar No. I seed in the year 1929. There was thus a strong circumstantial evidence in favour of seed dissemination. This was further confirmed by the occurrence of the disease on the Poona Agricultural College Farm, where wilt was previously absent, when a susceptible type of cotton seed from the wilt infested plots on the Jalgaon Farm, was introduced.

In the meanwhile work at the Cotton Research Laboratory at Dharwar was accumulating experimental evidence in support of seed-borne infection. In connection with innumerable pot culture experiments, it was noted that occasional stray plants died of wilt in the control series when every possible precaution was

taken against accidental infection. In the early period of the work (1924-26) death of about 160 seedlings was recorded as enumerated in the table below : –

TABLE IX.

Death of cotton seedlings recorded in the pot experiments of the control series for the years 1924-25 and 1925-26.

1924-25		1925-26	
Pot No.	Number of plants dead	Pot No.	Number of plants dead
		Series I.	
3	3	8	2
11	1	19	2
21	3	59	1
27	4	67	4
45	1	130	1
51	2	135	1
57	3	*Series II.*	
61	2	375	1
63	3	*Series III.*	
73	5	20	1
95	7	23	1
103	1	28	1
107	1	35	2
145	3	72	3
197	1	83	1
205	1	114	1
		130	3
		416	2
		425	2
		426	3
		Series IV.	
		7	1
		123	1
		131	1

All these dead plants showed typical wilt symptoms. Their xylum was either blackened or contained fungus hyphae.

This not infrequent occurrence of wilt from otherwise healthy plants led us to isolate the fungus from some plants to test its parasitism. Accordingly isolations from pot Nos. 19, 135 and 421 were made. The fungus from these isolations resembled the standard culture of *Fusarium vasinfectum* and gave positive proof of its being parasitic to cotton plants as seen from the results from Table X.

TABLE X.

Inoculation experiment with Fusarium vasinfectum *isolated from wilted plants from pot Nos. 19, 135 and 421 in the year 1925-26.*

	Inoculated		Control	
Fusarium isolation	Total plants raised	Plants wilted	Total plants raised	Plants wilted
From control pot No. 135 . . .	22	20	20	*Nil.*
,, ,, ,, No. 19 . . .	19	19	16	,,
,, ,, ,, No. 421 . . .	48	48	32	,,

The seed was delinted in sulphuric acid and was well washed with water and was sown in pots containing steam sterilised soil which was inoculated with the 15-day old cultures of the above isolations grown in Richards' medium. The controls were in sterilised soil without fungus.

Having thus been impressed with the possibility of the occurrence of such a source of infection, the investigation was continued.

Review of previous investigation.

Some of the *Fusarium* wilt disease have been known to be seed-borne. Bolley [1901] demonstrated that seed from wilt-affected flax plants was capable of producing disease in the crop. Since that time several workers have tried to connect the spread of wilt diseases with this agency. The death of scattered individual plants in the fields where there had been no wilt before has often tempted them to suspect the seed-borne nature of the disease. Orton [1931] lists nearly a dozen of *Fusarium* wilt diseases of crop plants, disseminated through seed.

In case of cotton wilt Elliot [1923] was the first to produce experimental evidence showing that wilt is carried in the interior of the seed. His results were later corroborated by Crawford [1923].

Recently definite proof of seed carriage of cotton wilt has been presented by Taubenhaus and Ezekiel [1932]. They obtained in one experiment 3·3 per cent. wilt attack in plants raised from seed collected from wilt-affected plants. In the second test 2·2 per cent. plants got diseased. They were also able to demonstrate the growth of *Fusarium* mycelium from the seeds when cultured on agar medium in Petri dishes.

Methods.

Laboratory tests.—The first tests at Dharwar were made in 1924-25 following the Petri dish method of Elliot. The seed for the experiment was collected from plants grown in heavily wilt-infested land. It was treated with strong sulphuric acid for five minutes and was then well washed with sterile water and was immediately placed in sterile Petri dishes containing filter paper dipped in rice starch. Many Fusaria by their action change the white colour of starch to violet blue, a fact which makes it easy to locate the fungus. The experiments were started on the 21st of November 1924 and terminated with the month of May 1925. Within that period 7,275 seeds were tried. In Table XI the data represent the days on which the seeds were placed in Petri dishes. Each trial lasted for fifteen days.

TABLE XI.

Results of seed germination test to see if it carries infection of the wilt disease.

Date	No. of dishes	No. of seeds	Results
21st November 1924	50	500	No fungus.
5th January 1925	60	600	No fungus.
9th „ 1925	55	550	No fungus.
20th „ 1925	60	600	No fungus.
3rd February 1925	50	500	No fungus.
11th „ 1925	50	500	No fungus.
22nd „ 1925	55	550	No fungus.
27th „ 1925	47	475	No fungus.
10th March 1925	50	500	No fungus.
2nd April 1925	57	575	No fungus.
24th „ 1925	62	625	No fungus.
29th „ 1925	67	675	No fungus.
4th May 1925	47	475	No fungus.

The data recorded in the above table show that out of 7,275 seeds not a single one showed the presence of wilt *Fusarium*. It may, however, be remarked that these tests were in the nature of preliminary trials and were not satisfactory in certain respects. There being, at that time, no special culture room, the experiments had to be carried out in the working room of the laboratory. And it, therefore, became very difficult to exclude the ordinary moulds which soon overran the dishes and probably prevented the *Fusarium* fungus growing out of the seed.

Later attempts of culturing the seeds in 1932-33 have been of success. Out of 1,100 seeds 22 gave rise to *Fusarium* growth, which on isolation agreed with the standard culture.

Pot culture experiments.—Seed was obtained in the season 1928-29 from plants showing typical symptoms of wilt. The plants had most of their branches killed and had their internal wood of stem and root blackened ; and such parts on incubation produced *Fusarium* fungus. The seeds before being sown in pots (each 6 inches in diameter and 6 inches deep) containing sterilised soil was delinted in commercial sulphuric acid and was well washed in distilled water. The plants were given optimum conditions for wilt development (this was the method adopted in all the trials mentioned hereafter). In a trial of 1,087 seeds 30 seedlings died of wilt, thus giving 2·8 per cent. attack for the trial.

In the season 1929-30 it was decided to study the problem more critically. In order to do it, instead of the seed being collected at random from diseased fields, ripened bolls were secured from plants showing definite evidence of infection, *viz.* :— the fungus could be traced right from the root to the stem branch and entering into the fruiting branch. The pedicels of such bolls were examined in the laboratory for fungus infection either by sectioning the piece for observation under the microscope or by incubating it in the moist chamber for fungus growth. Out of 600 bolls collected 82 bolls showed evidence of the presence of the fungus in the pedicel. When 298 seeds from 28 such bolls were grown wilt developed in 17 seedlings. It was thus shown that though the fungus is carried inside the affected plants in a good many cases it fails to reach the pedicel and in very few cases only does it enter the seed.

In the year 1930-31 there were 238 bolls showing the presence of the fungus in their pedicels. Out of these 11 bolls showed evidence of wilt in their seeds. The total number of plants raised was 2,685. The data of all these experiments are noted below :—

TABLE XII.

Experiment 1.

Development of wilt in seeds collected from wilt-affected plants.

Year	Number of plants raised	Number of plants wilted	Remarks
1928-29	1,087	30	....
1929-30	298	17	From 28 bolls.
1930-31	2,685	22	From 11 bolls.

Having thus obtained definite evidence as to the disease being carried inside the seed in the Karnatak tract, it was decided to study the problem with seed obtained from other wilt tracts in the Bombay Presidency. Seed was obtained from Broach Farm where wilt is very common. The trials as noted below have borne out the previous results.

TABLE XIII.

Experiment 2.

Development of wilt in seeds of cotton (A long boll) from Broach Farm.

Number of trials	Number of plants raised	Number of plants wilted	Wilt percentage
1	276	61	22·1
2	595	43	7·1
3	145	4	2·8
4	169	3	1·8
5	62	13	20·9
Total .	1,247	124	9·9

Out of the total number of seeds 1,247 tried in the above trials, 124 seedlings developed wilt and this when worked out on percentage basis comes to 9·9, a figure much greater than in any of the previous trials.

In the season 1931-32 seed was obtained from other wilt prevailing areas such as Jalgaon (Khandesh), Akola (Berar), and Cawnpore and Raya (United Provinces). The results which again support the previous ones are noted below. A few of the pots are shown in Plate LXVIII.

TABLE XIV.

Development of wilt from seed from wilt-affected cotton plants obtained from Jalgaon, Akola, Cawnpore and Raya in the year 1931-32.

Place from which seed was obtained	Number of plants grown	Number of plants wilted	Wilt percentage
Jalgaon . . .	677	48	7·0
Cawnpore . . .	198	33	16·7
Raya	138	2	1·4
Akola	608	5	0·72

Field trial.—In addition to these pot experiments field trial was made in the year 1931-32. A small triangular plot where no wilt was observed in previous years, was sown with seed collected from diseased plants. Out of the total number of 3,280 plants grown, 110 wilted.

Discussion.

The results of all the above trials purport to show the seed-borne character of the wilt fungus. Though the fungus itself has not been observed in the seed the evidence presented strongly points to such a character. It may be noted that, in the experiments, all possible precautions were taken to avoid accidental infection. The seed was surface-sterilised in sulphuric acid and was sown in pots containing sterilised soil. If such a seed produced wilt it was but natural to surmise that the infection was carried within the seed, since all the outward infection of the seed was destroyed by the acid treatment. This surmise has been borne out by the isolation of the wilt fungus from such seeds when cultured on agar medium in Petri dishes.

Evidence has been presented to show that though the fungus is carried inside the affected plants, in a good many cases it fails to reach the pedicel and in very few cases only it enters the seed. In a general organisation of seed distribution where large quantities of seed are handled, the possibility of disease being carried by seed might seem very small. The very few individual seeds, however, serve as starting points of infection and may thus become a potent source of danger for any wilt-free area, suited to the establishment of disease.

As will be seen in the next chapter the fungus is killed when exposed for 30 minutes to a temperature of 60°C. This is moist heat because the fungus is grown in liquid medium. This fact might be taken advantage of to kill the fungus inside the seed, by the hot water method. But it is doubtful whether such a treatment will not injure the vitality of the cotton seed. It is not known whether the above temperature might be efficacious to destroy the fungus in the seed in dry conditions. In case of cotton anthracnose Lehman's [1925] tests of effect of heat on cotton seeds show that if the seed is dried slowly it will stand 70°C. for many hours (up to 72) and even 90°C. if carefully predried. Massey [1929] also did a good deal of work on heating cotton seed with 'black arm disease' of cotton. He obtained promising results by subjecting the cotton seed for five hours to dry heat at 95°C. Dry heat has been used in the disinfection of seed of the cereals such as wheat, barley, rye and oats. It has been found that when cereal seeds are of good quality and well dried, they will withstand protracted exposure to dry heat at 100°C. for 30 hours. Investigation is therefore necessary to elucidate all these points with regard to cotton seed.

Chapter IV.

Relation of soil temperature and other factors to the development of cotton wilt.

The study of the pathogenicity of *Fusarium vasinfectum* Atk., the causal organism of the cotton wilt disease [Kulkarni, 1928], gave certain indications that the development of the disease in the crop is conditioned on certain environmental factors. It was noticed that disease in the plants could be brought about by inoculating with the wilt fungus the soil in which the plants grew, for a certain period of the year, after which attempts to develop infection in the plants with all possible means completely failed. It is a matter of general experience that the development of disease in plants is considerably influenced by environmental factors irrespective of the pathogenicity of the parasite and the susceptibility of the host. The regional and seasonal fluctuations in the occurrence and the severity of the disease which were difficult to understand hitherto are now being explained through the critical study of these factors and indeed the study has already been of such value as to permit of some application of its results to disease control. The practical importance of this study, therefore, being thus evident an investigation was undertaken to elucidate some of the factors which favour or hinder the development of cotton wilt. The following soil factors were studied. (1) Temperature; (2) moisture; (3) the nature of the soil and its reaction; (4) organic matter and (5) sowings at different periods in the season.

In studying these factors the following procedure was adopted. Experiments were made in pots filled with sterilised (autoclaved at 30 pound pressure for 30 minutes) soil which was artificially inoculated with the wilt fungus grown in Richards'* liquid medium. The seed used was of Dharwar No. 1, a type very susceptible to wilt and was delinted in sulphuric acid before sowing. Controls were in sterilised uninoculated soil.

Temperature.

Of all the soil and climatic factors which affect crops and their predisposition to disease, temperature perhaps is the most important. Since the introduction of "Wisconsin soil temperature tanks" the influence of this factor on disease incidence

*Richards' medium :—

Potassium nitrate	10 grms.
Acid potassium phosphate	5 ,,
Magnesium sulphate	2·5 ,,
Cane sugar	30 ,,
Distilled water	1,000 c.c.

has been prominently brought to the notice of workers in the field of plant pathology. It has been shown by Jones and others [1926] for a number of diseases, including several *Fusarium* wilt diseases, what a dominant part temperature plays in the development of disease in crops.

In the early period of the present investigation, *i.e.*, in the year 1925-26, it was noticed that marked difference existed in the rate of wilting of susceptible cotton plants grown in pots containing infested soil. Plants were raised in successive months throughout the season. The table below gives the data obtained.

TABLE XV.

Development of wilt in Dharwar No. 1 cotton seedlings grown in pots containing infested soil during the season, 1925-26.

Period of experiment	Temperature at 6-in. depth of soil during the period (°C).		Date of sowing	Final dates when readings were taken	No. of plants grown	Number wilted	Wilt percentage
1925.	8 a.m.	4 p.m.					
August-September .	23	26	17th Aug. '25	10th Sept. '25	27	27	100·0
September-October .	24	30	11th Sept. '25	10th Oct. '25	22	13	59·0
October-December .	20·5	28·4	8th Oct. '25	12th Oct. '25	21	16	76·0
November-January .	20·5	24·5	8th Nov. '25	12th Jan. '26	39	35	89.0
December-February .	20·5	28·3	16th Dec. '25	12th Feb. '26	20	8	40·0
1926.							
January-February .	23	28·4	13th Jan. '26	21st Feb. '26	29	25	87·0
February-March .	24	31·6	12th Feb. '26	6th Mar. '26	29	..	..
March-April . .	26·5	32·0	9th March '26	6th April '26	29	..	..

The evidence presented in the table shows that wilt is very aggressive when the maximum temperature is below 26·6°C. (August-September and November-January); it decreases in severity as the temperature rises above 26°C. and completely stops as soon as the temperature reaches near 32°C. (February-March and March-April). This irregular behaviour of the disease suggested the possibility of its relation to the soil temperature. In the following seasons (1926-27, 1927-28 and 1928-29),

therefore, field observations on the incidence of wilt in the crop were started and records of soil temperature at 6-inch depth by a soil thermograph were also obtained. The observations were made on the crop sown with seed susceptible to wilt, in a field known for its high infection. In the season 1926-27 and 1927-28 there were four replications and each replication consisted of four lines and the number of plants for each season was 708 and 679. In 1928-29, there were six replications of five lines each and the plant number was 1,523. Weekly wilt percentages were recorded. The temperature figures were the weekly mean maxima obtained by adding the daily maxima for each week and dividing the sum by 7. The data for the three seasons are represented as shown below.

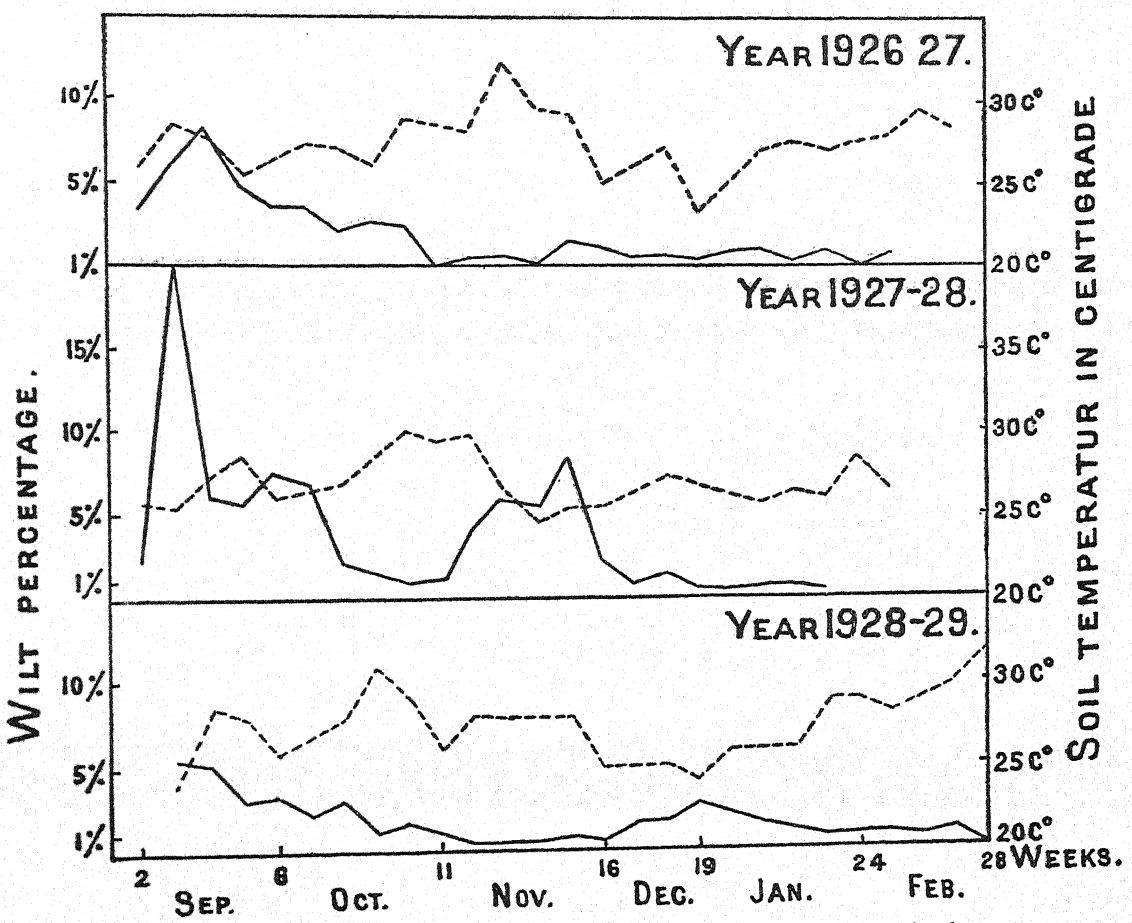


Fig. 1. The relation of soil temperature to wilt during the cotton growing season for the years 1926-27, 1927-28 and 1928-29.

It is seen that in all the three graphs, in the beginning, wilt is rather high consequent upon the low temperature which is invariably below 25°C. The wilt curve then begins to fall reaching its lowest ebb by about November in the two seasons, 1926-27 and 1928-29, while in the season 1927-28 this occurs in October. In contrast to this the temperature curve has gone up, reaching to about 30°C. It then falls coming down below 25°C. when the wilt curve again rises. It is thus clearly shown that temperatures of 25°C. and below favour wilt and higher temperatures retard it.

The pot culture experiments described early in the paper have shown that a temperature above 32°C. completely inhibits wilt. It was thought that successive field sowings of cotton in small plots throughout the year might show considerable variation in the wilt attack. It seemed possible that during the months of March, April and May when the mean maximum soil temperature is high (above 32°C.) wilt might be completely inhibited or materially checked and during the months of June and July when the temperature comes down considerably (below 25°C.) wilt might be greatly stimulated. In order to test this out, a series of plantings were started at intervals of 30 days, throughout the year, from January to December. Plants were raised in small plots (8ft. × 3ft.) in a field heavily infected with the disease. Weekly wilt percentages were obtained and the average for the month was deduced by adding the weekly figures and dividing the sum by the figure representing the number of weeks in a month. These computations together with those (mean monthly maximum) of soil temperature at 6-inch depth recorded by a thermograph are represented graphically in the following figure.

RELATION OF TEMPERATURE TO WILT DISEASE.

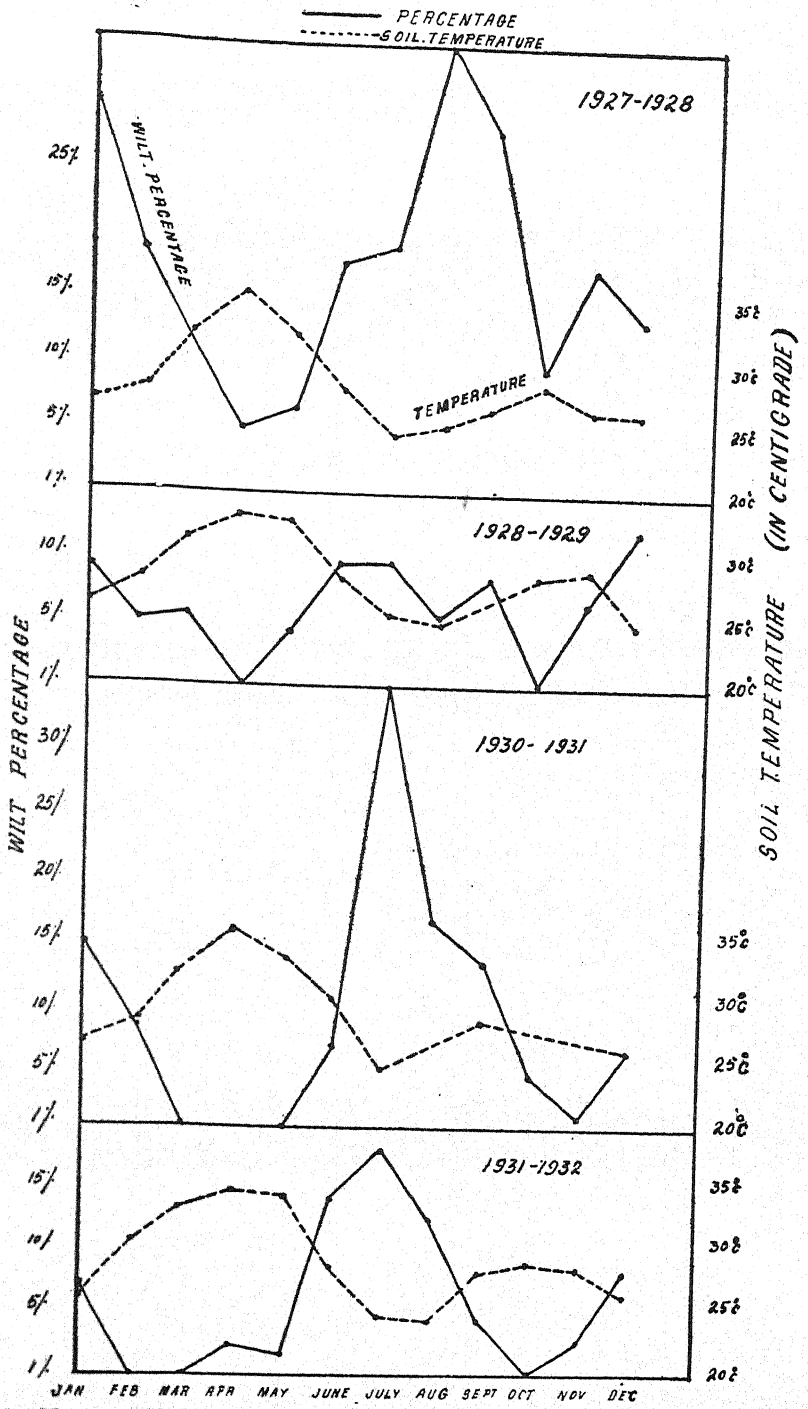


Fig. 2. Mean monthly maximum soil temperature at 6 in. depth as it occurred in "successive sowings" plots, and the development of wilt in one month old cotton seedlings in the same plots for the years 1927, 1928, 1930, 1931.

In all these figures it can be seen that there is about 5 per cent. wilt in the crop to start with, *i.e.*, in January, because of the favourable soil temperature (about 25°C.) for wilt development. Wilt then gradually decreases due to the rise of temperature and comes to a stand still in April (see figure for the year 1928-29 and 1930-31), when the temperature goes beyond 32°C., a point inhibitive to wilt development. Then it begins to rise again and reaches its maximum in July when the temperature for the year is the lowest, *i.e.*, the optimun for wilt production. There is again a fall sometime in October consequent upon the second rise of temperature. A correlation between the soil temperature and wilt development is thus clearly seen.

These field observations have been further checked under controlled laboratory conditions in pot culture experiments. The trials were done in special temperature tanks wherein the temperature once adjusted to any range remained pretty constant. With regard to the control of temperature in these tanks it was easy to manipulate so long as the desired temperatures were above the maximum air temperature which at the time of working these experiments varied between 25 and 27°C. To get temperatures lower than 26°C. the tanks could not be made use of as there were no facilities to get ice to run cold water through the tanks. A special device had to be used by which temperatures lower than air could easily be secured. It consisted of keeping the metallic pot containing the plants in an ordinary earthen pot full of water which got cooled due to its constant evaporation and consequently the desired lower temperature was obtained (Plate LXVIII). It ranged from 20 to 25°C.

The heating arrangement of the tank was like an ordinary incubator by an oil lamp; each tank had ten metal containers to grow plants. The soil used was the field soil which was autoclaved for 30 minutes at 30 pound pressure. The inoculum used was fifteen days old culture of wilt fungus grown in liquid medium (Richards') in a big four-litre flask. The mixing of the culture in the soil was done 15 days in advance of starting the experiments. Seeds of Dharwar No. 1 cotton, susceptible to wilt, were sown soon after the tanks were adjusted to the required range of temperature. After germination the seedlings were thinned, retaining only the desired number in each container. The soil moisture in the experiments was such as to ensure a good growth of plants and was kept reasonably constant by frequent waterings. The numerical results of this experiment are represented below.

TABLE XVI.

The relation of soil temperature from 20 to 35°C. to the development of cotton wilt in Dharwar No. 1 cotton in artificially inoculated soil.

Duration of experiment	Temperature (°C.)	Inoculated soil		Uninoculated soil	
		No. of plants	Plants wilted	No. of plants	Plants wilted
	Test I.				
1st July 1930 to 1st August 1930	20 to 25	27	27	13	0
	26 to 27	40	40	12	0
	30 to 31	31	28	10	0
	31 to 32	34	21	11	0
	Test II.				
30th August 1930 to 29th September 1930.	20 to 25	66	66	69	0
	26 to 28	65	54	64	0
	29 to 31	63	44	64	0
	30 to 32	69	36	69	0
	Test III.				
11th October 1930 to 21st November 1930.	22 to 26	82	52	12	0
	26 to 30	102	64	11	0
	29 to 31	75	22	12	0
	30 to 34	65	0	13	0
	Test IV.				
10th March 1931 to 1st May 1931	22 to 27	87	79	17	0
	32 to 37	55	0	13	0

At each of the soil temperatures in test I, four cans were used out of which three were for inoculated soil and one for control. There was considerable difficulty in maintaining the temperature of the tank adjusted to 26-27°C. Due to the

prevalence of continuous cool weather the temperature of the tank went below 25°C. on several nights and consequently the results are vitiated. Wilt symptoms began to appear first at 25°, 27° and 31°C., 12 days after planting, while at 32°C. they were manifest after 20 days. At the end of the experiment all plants at 25° and 27°C. were dead, while at 31° and 32°C. there was a gradual reduction in wilt. No disease occurred in the control.

In test II, five cans were for the infested soil and five for control and therefore the number of plants raised was greater than that in test I. Here too cent. per cent. wilt occurred at 25°C. At 28°C. there was a decided check. The higher temperatures of 31° and 32°C. had a corresponding decrease in wilt. The controls were again free.

In test III, for each temperature there were nine cans for inoculated soil and one for the control. The range of temperature for each tank in this test was slightly wider than in previous ones. A clear limitation in infection can be noticed at 26°C. against 25°C. in tests I and II. The same effect was noticeable between 30° and 32°C. Sixty-four out of 102 wilted at 30°C. as against 22 out of 75 at 31°C. Beyond 32°C. no infection occurred. Controls as usual were free.

In test IV, the reduction of wilt at 27°C. and its complete cessation at above 32°C. was again manifest.

There is some evidence to believe that cotton wilt is a low temperature disease, manifesting itself severely between 20° and 25°C., decreases with the rising temperatures (26° to 31°C.) and stopping completely beyond 32°C. At lower temperature (below 20°C.) the disease might fall off gradually but the actual minimum has not been determined.

The question then arises whether the inhibition of infection at high temperature (32°C. and above) is expressed in effect upon the parasite and host separately or jointly. It might be that the fungus at increased temperatures is affected in its growth or loses its virulence or the host gets changed in constitution to render it more resistant to the parasite than at the lower temperatures. In order to investigate this problem a study of the influence of temperature on the growth of the fungus and the host was undertaken.

Influence of temperature on the growth of the fungus.—The influence of temperature on the growth of the fungus has been worked out. The fungus was grown on Richards' medium in 50-c.c. flasks which contained 15 c.c. of the medium. After seven days' growth the dry weight of the mycelium formed was determined in the usual way.

TABLE XVII.

Results showing the dry weight of the mycelium of the wilt fungus grown seven days in Richards' medium at different temperatures.

Temperature	39 to 43°C.	34·5 to 38·5°C.	32 to 34°C.	28·5 to 32°C.	27·5 to 30°C.	26 to 28°C.	24 to 26°C.	23 to 25°C	21 to 23°C.	19 to 20°C.	17 to 18°C.	13 to 15°C.
Dry weight of the mycelium in grams.	No growth.	·0038	·1000	·1121	·1139	·1193	·1182	·1150	·0424	·0182	·0020	·0012

A duplicate flask kept at temperature 39° to 43°C. for five days when transferred to 24°-26°C. temperature did not show any growth after five days. Practically no growth occurred at 13 to 15°C. and at the high temperature 39° to 43°C. The maximum growth was between 26° and 28°C. The thermal death point for the fungus is above 40°C. The growth temperature relation of the fungus therefore ranges from 13°C. to 43°C. with an optimum somewhere between 26 and 28°C.

In this connection it is interesting to note the results of other workers on the relationship of soil temperature on the development of wilts caused by various species of *Fusaria* on crops like tobacco, tomato, cabbage and flax. In all these studies the optimum temperature for the growth of these fungi in culture and for the development of disease in their respective hosts has been found to be somewhere near 28°C. With regard to cotton wilt according to Young [1928] the coincidence has been shifted by two degrees and centers round 30°C. (Table XVIII). Fahmy [1931] found the cotton wilt fungus growing best between 30 and 35°C.

TABLE XVIII.

Comparison of the optimum temperatures for the growth of the organisms in pure culture and for the development of disease caused by them in their respective hosts.

Host	Organism	Temperature (°C.)		Authority
		Optimum for organism	Optimum for disease	
Tomato . .	*Fusarium lycopersici* . .	28	28 to 29	Jones, Johnson.
Tobacco . .	*F. oxysporium* var. *nicotianae*	28 to 30	28 to 31	
Cabbage . .	*F. conglutinans* . . .	25 to 27	26 to 29	Jones *et al* (1926).
Flax . . .	*F. lini*	24 to 28	24 to 28	
Cotton (U. S. A.) .	*F. vasinfectum* . . .	30	28 to 32	Young (1928).
Cotton (India) .	*F. vasinfectum* . . .	28	20 to 25	

From this it has been concluded "that the influence of temperature upon disease development (that of *Fusarium* wilt disease in general) is primarily due to its direct effect on the parasite". With cotton wilt, however, as studied at Dharwar the optimum (20° to 25°C.) for the disease lies quite below that (28°C.) for the fungus on agar medium as seen in Fig. 3 below.

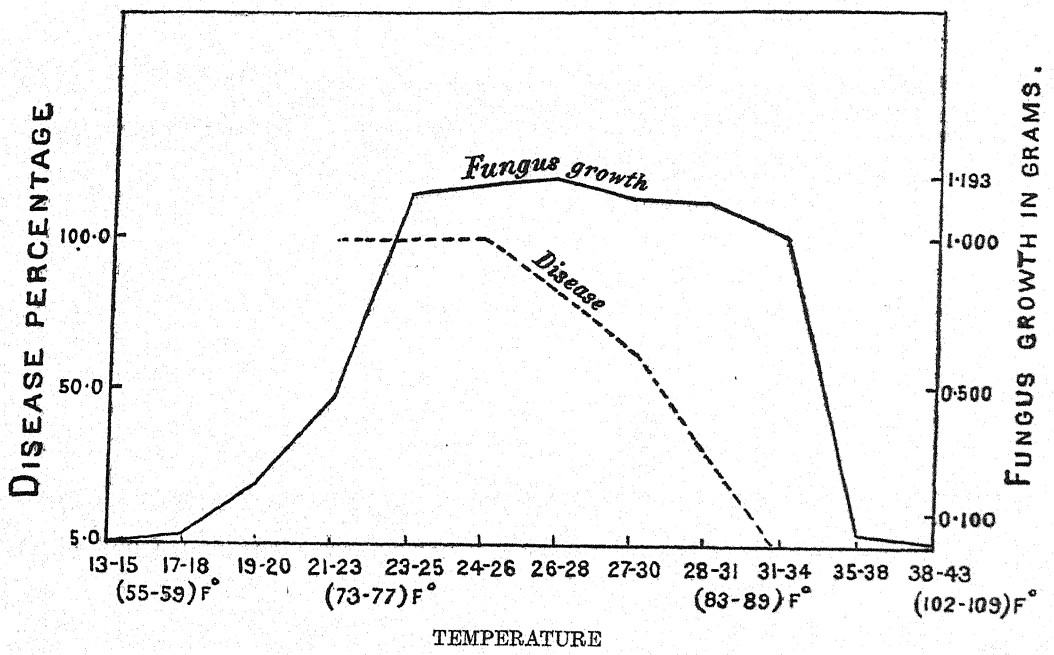


Fig. 3. Graphic comparison of the influence of incubator temperatures (°C.) upon the growth of the fungus, *Fusarium vasinfectum*, with that of the corresponding soil temperatures upon the development of the cotton wilt disease caused by it.

There appears, therefore, very little connection between the rate of growth of the fungus and its pathogenicity. The retardation of wilt at a temperature of 28°C., which induces the greatest growth of the fungus in culture, and its abrupt inhibition at 32°C. when the fungus is still active probably indicates that the influence of soil temperature is mainly on the host.

Influence of temperature on the growth of the cotton seedlings.—No detailed study of the effect of temperature on the growth of the cotton plant has been made at Dharwar but observations on the effects of soil temperature on the germination and growth of cotton seedlings for about two months have been recorded. At 20°C. germination starts very slowly, as it takes 8 to 10 days for the seedlings to appear above the soil. They remain poor in appearance and are not more than four or five inches in height at the end of two months. At 25°—27°C. germination is

considerably quickened and is over in four or five days. At 30°—35°C. it takes only two days for the seedlings to appear. Plants get sturdy and put on a vigorous growth. Temperatures above 35°C. seem to be unfavourable for the cotton plants. At 40°C. although a very quick germination is secured the plants grow very slowly and remain in a stunted condition even after two months. The optimum temperature for the cotton plants, therefore, appears to be somewhere between 30° and 35°C. These results agree with those of Balls [1919] who states 30°—35°C. to be the most favourable temperature for the development of cotton. According to Camp and Walker [1927] the optimum temperature for cotton seed germination is between 33° and 34°C. Young [1928] secured finally good growth of cotton plants between 25° and 35°C. It therefore appears that at 32°C. when the disease abruptly stops it is not due to the decreasing activities of the fungus but to the most vigorous growth of the host as seen above. May not then the reaction of the cotton plant as manifested by its susceptibility, resistance and immunity at different temperatures be evidently due to the direct action of temperature on it ?

Geographical distribution of cotton wilt in India.—The study of soil temperature in relation to wilt seems to throw some light on the limitation of the disease to certain tracts. It is more or less common in the Bombay-Karnatak, Khandesh and Berar and occurs to a limited extent in South Gujarat. It does not appear at all in North Gujarat, Sind and in the Punjab but is found sparingly in certain parts of the United Provinces. This seems to be in harmony with experimental results if the climatic conditions are concerned. The average maximum temperature of the wilt prevailing tracts at the cotton growing season (June to February) ranges from 22° to 28°C., and this is the most favourable temperature for the development of wilt. On the other hand the disease gets less at 30°C. and stops completely at 32°C. and above, which is the mean temperature (maximum) of the Northern Indian cotton season (April to October) and that explains the complete absence of wilt in the Indo-Gangetic plain.

Trial of control measures suggested by the temperature studies.—As noted previously the thermal death point of the fungus is above 40°C. Under laboratory conditions an exposure to 40°—42°C. for four days is required to kill the fungus. At higher temperatures, however, the time required is considerably decreased. At 56°C. an exposure of 2—2½ hours is sufficient while at 60°C. only 30 minutes are required. It has also been found that the temperature of the surface one-inch layer of the soil in the field reaches 60°C. in the hot months of March-April and May. In fact the temperature remains above 60°C. for four hours in the middle of the day. These results indicate that by a suitable system of cultivation in March-May (during

which period the cotton crop is off the ground) the temperature of the surface soil could be raised sufficiently to kill out the fungus, thus suggesting a practical method of control. An experiment was therefore tried for two seasons in the field plots noted for wilt infection. The treatment consisted of ploughing the land to a depth of 6 inches every week so as to turn over the soil every time in the hot season beginning from the month of March to the end of May. In the season 1927-28 the experiment was tried on three plots, with an area of 7 *gunthas* each. Two were for treatment and one was for control. In the season 1928-29 the plots chosen were not of uniform wilt infection. In order therefore to reduce the error of experimentation each plot was marked for high and low wilt infection. Half of each marked plot was treated and the other half was control.

TABLE XIX.

Results showing the percentage of wilt in the cotton sown in plots with hot-weather treatment.

Treated				Control			
Plot No.	No. of Plants	Plants wilted	Wilt percentage	Plot No.	No. of plants	Plants wilted	Wilt percentage
Season 1927-28.							
381	2441	1493	61·1	385	864	619	71·7
382	2485	1273	51·1	..	..	..	..
Season 1928-29.							
High wilt area.							
367	753	552	73·3	367	638	393	61·9
375	735	311	42·3	375	629	275	43·7
Low wilt area.							
367	738	398	53·8	367	730	341	46·7
375	740	364	49·2	375	721	242	33·3

The results do not seem to show any advantage of hot-weather treatment in reducing the wilt attack in the crop. The failure of these experiments to kill out the fungus from upper layers of soil necessitated the determination of the depth to which the fungus is found in the soil. The experiments were done in pot cultures. In order to imitate the field conditions, soil at different depths of each layer 4 inches from the infected field was placed in its respective place, the other layer or layers in the pot being sterilised (autoclaved) soil. The total depth of soil in each pot was 20 inches.

TABLE XX.

Results of pot culture experiments to show the depth to which the wilt fungus is found in the soil. There were five pots for each layer.

Duration of experiment	Depth of soil (in.)	No. of plants grown	Plants wilted	Wilt percentage
10th January 1928 to 25th October 1928	1st 4	74	63	85·1
	2nd 4	77	58	75·3
	3rd 4	72	41	56·9
	4th 4	73	31	42·4
	5th 4	75	26	34·7
		Duplicate		
	1st 4	73	67	91·7
	2nd 4	74	66	89·2
	3rd 4	71	60	84·5
	4th 4	72	56	77·8
	5th 4	72	59	81·9
12th October 1929 to 1st January 1930	1st 4	69	46	68·1
	2nd 4	69	57	82·6
	3rd 4	62	49	79·0
	4th 4	61	50	81·9
	5th 4	69	51	73·9
	1st 8	139	94	67·6
	2nd 8	127	92	72·4
12th August 1930 to 1st October 1930	1st 4	88	67	64·8
	2nd 4	92	73	79·3
	3rd 4	94	69	73·4
	4th 4	87	52	59·8
	5th 4	92	50	54·3
	1st 8	166	127	76·5
	2nd 8	190	124	65·2

It is evident that the fungus is distributed pretty deep in the soil up to 20 inches thus accounting for the futility of hot-weather cultivation.

Moisture.

The effect of soil moisture on the parasitic attack or organisms causing wilt seems to be of considerable importance. Some organisms are particularly virulent under high soil moisture content while others seem to be more active with low soil moisture contents.

Clayton [1923] showed that tomato plants growing in a low soil moisture content were resistant to wilt, while rapidly growing plants with optimum moisture conditions succumb quickly.

With Thielavia root-rot of tobacco Johnson's [1919] observations indicate that the saturation of the soil greatly increased the amount of disease.

The studies of Johnson [1921] on tobacco wilt prove that excessive soil moisture retards disease.

Tisdale [1923] found that wilt in cabbage seedlings is very destructive when the soil moisture is rather low.

In the foregoing experiments on temperature the soil moisture maintained was such as to promote good growth of the plants. No attention was paid to its influence on disease when different quantities were applied. The following experiments were planned in order to study this question.

Experiments to determine the influence of this factor upon the incidence of wilt were started in the year 1925-26. The soil used for the experiment was from a wilt-infected field and its water-holding capacity was found to be 75 per cent. on the dry-weight basis. Glazed earthen pots 9 inches in diameter and 12 inches deep without any drain hole, were used for the trials. Four different percentages of moisture ranging from 27 to 70 were tried. The number of trials made was three. In the first and the second trials 27, 40, 60 and 70 percentages of moisture were maintained. In the third the percentages were 27, 40, 50 and 60. For each percentage there were four pots, two for treatment and two for control. Thus there were 16 pots for each set of experiments. The soil before being potted was mixed with 20 per cent. farmyard manure and was autoclaved at 30 pounds pressure for 30 minutes and was then inoculated with the fungus culture grown in Richards' medium. Each pot received 20 lbs. of soil. In order to obtain uniformity in soil-moisture which is said to depend upon the compactness of its mass, a uniform pressure was applied to the soil while being packed into the pots. Seed was sown a week after the pots were ready with the soil and the moisture was made up to the required

percentage after the completion of germination. The pots were weighed daily during the progress of the experiment and water was added through a bamboo tube buried in the pot to replace the losses. In order to prevent rapid evaporation of the surface soil a thin layer of sand was maintained on all the pots. The data of these trials are given below :—

TABLE XXI.

Relation of soil moisture to the incidence of wilt.

Inoculated series I				Inoculated series II			Inoculated series III			
Treatment (per cent.)	Total plants	Deaths	Death (per cent.)	Total plants	Deaths	Death (per cent.)	Treatment (per cent.)	Total plants	Deaths	Death (per cent.)
27	33	21	63·6	33	8	24·2	27	32	21	65·6
40	42	26	61·4	36	24	66·7	40	33	18	54·5
60	34	16	47·1	32	29	80·6	50	34	31	91·1
70	36	19	52·8	28	17	60·8	60	21	19	90·5

There does not seem to be any effect of these variations of soil moisture on wilt as there is no regularity in the results.

Moisture determinations at 6-inch depth of the cotton fields during the entire season beginning from the month of August when the crop is sown, to the end of February when the harvest begins, are shown below.

TABLE XXII.

Moisture determination at 6-inch depth of the soil during the period when the cotton crop is in the fields.

Months	Percentage of moisture
August	32·6
September	31·1
October	34·1
November	30·8
December	26·6
January	20·6
February	18·0

It is seen from Table XXII that the initial soil moisture for the crop is 32·6 and reaches its maximum (34·1) in October and then gradually comes down reaching the lowest limit of 18 per cent. in February. It seems that the soil moisture does not go above 35 per cent. even in the wettest part of the season. The lower soil moisture percentages tested 25 and 27 (Table XXI), are much below 32, the initial soil moisture for the crop, and the percentages 40, 50, 60 and 70 are much higher than 34, the maximum found in the field condition. Notwithstanding this wide range of variations as obtained in pot culture (Table XXI), there is no influence of moisture on wilt. It may, however, be noted that the condition of the plants in 40 per cent. and 50 per cent. moisture was the best of all. The plants were vigorous and tall and produced flowers freely as compared with those in 27, 60 and 70, which were weak and stunted due either to too low or excessive moisture.

The effect of varying the quantity of moisture on wilt having been not definite it was thought that pot culture experiments may not reproduce filed conditions and it was decided that trials should be done in small blocks in the field. Accordingly five beds each 7 feet by 4 feet were prepared. The four sides of each bed were protected by iron sheets buried two feet deep in the soil while the bottom layer was quite free. The percentage of moisture to be maintained in each bed was as under :—

First block 60 per cent.
Second block 50 ,,
Third block 40 ,,
Fourth and 5th blocks (control).

To start with, moisture in the field was 35 per cent. In each bed the quantity of water required to make up the percentage was added every day and the determinations of moisture were done the next day. The experiment was started on the 13th October 1926 and was over on the 31st January 1927.

The details of this experiment are tabulated below :—

TABLE XXIII.

Controlled moisture experiments in field conditions on the incidence of wilt.

Block No.	Moisture percentage to start with	Percentage of moisture to be maintained	Quantity of water required to be added daily (lb.)	Percentage of moisture found next day	Plants grown	Plants wilted	Wilt percentage
1	35	60	1,200	40 only	97	66	68·1
2	35	50	680	40	107	69	64·5
3	35	40	200	40	102	66	64·7
4	35	35	*nil*	35	116	68	58·6
5	35	35	*nil*	35	137	81	59·1

It is to be noted that irrespective of the quantity of water added to make up the required percentage in each bed the percentage was never above 40 next day, indicating that extra water drained away underneath. It being therefore impossible to maintain the moisture in the soil above 40 per cent. the observation had to be made on this quantity of moisture only. Nor could the lower percentages, such as 30 and 25, be obtained along with the 40 per cent. bed, as the initial moisture was 35 per cent. and this percentage fell so very slowly that by the time it came down to 25 per cent. most of the plants in the 40 per cent. bed were dead.

It is thus obvious that soil in these parts is not water-logged. Nevertheless a temporary water-logging for a few hours in the surface soil may still occur during rainy days and in order to see its effect on wilt, ridge *versus* flat sowing of cotton in the fields was tried for two seasons, 1928-29 and 1929-30. Plants one foot apart were grown on ridges 9 inches high and three feet apart, as against plants grown in the flat system where the distance between the plants and rows was the same as in ridge system. As seen from the results in Table XXIV the ridge system has no superiority over the flat sowing. On the contrary it seems to encourage wilt.

TABLE XXIV.

The effect of ridge versus *flat sowing on wilt.*

RIDGE SOWING			FLAT SOWING		
Total plants	Plants wilted	Wilt percentage	Total plants	Plants wilted	Wilt percentage
1928-29					
309	134	43·3	308	48	15·5
1929-30					
268	194	72·7	254	132	51·8

Since the discovery of the optimum temperature (20—25°C.) for the wilt development and the special technique evolved to grow plants under that temperature as described on page 1008 a second attempt was made to test the effect of variations of moisture on wilt, keeping temperature (optimum for wilt) constant, in pot cultures. The pots used in this experiment were small and handy, 6 inches in diameter and 6 inches deep. The procedure followed in the experiment was the same as described for the first experiment on page 1016. Pots were weighed once in 24 hours and the lost water was made up to keep the percentage of moisture constant. The results are tabulated on the next page.

TABLE XXV.

Influence of soil moisture on the development of wilt in Dharwar No. 1 cotton in soil inoculated with pure culture of wilt fungus, grown for two to three weeks at a soil temperature 25°C. optimum for wilt development.

Soil moisture (per cent.)	Duration of experiment 15th Aug. 1929 to 2nd Sept. 1929			Duration of experiment 9th Sept. 1929 to 27th Sept. 1929			Duration of experiment 12th Oct. 1929 to 6th Nov. 1929			Duration of experiment 14th Jan. 1930 to 4th Feb. 1930		
	Plants grown	Plants wilted	Wilt percentage	Plants grown	Plants wilted	Wilt percentage	Plants grown	Plants wilted	Wilt percentage	Plants grown	Plants wilted	Wilt percentage
25	29	28	96·5	38	35	92·2	42	42	100·0	29	29	100·0
30	27	21	77·8	35	29	82·9	38	37	97·4	33	28	84·8
35	32	28	87·5	37	35	94·6	40	38	95·0	27	25	92·6
40	30	29	96·6	32	32	100·0	40	40	100·0	32	32	100·0
45	31	26	86·1	31	24	77·4	40	38	95·0	32	29	90·6
50	33	31	93·9	38	35	92·1	37	33	89·2	35	29	82·9

These experiments on the influence of soil moisture upon the development of cotton wilt conclusively prove that soil moisture is not an important controlling factor in the prevalence of cotton wilt.

Soil types and their reaction.

It is common to find wilt-free field in the tract where wilt is more or less common while some areas such as those of the Gokak Farm are remarkably free from wilt. In order to see whether such soils owe their freedom from wilt to any special cause, tests were made with these soils. They were steam-sterilised before being artificially infected with the wilt fungus. The kinds of soil used for the experiment were (1) Dharwar soil, a typical black clay soil known for its wilt infestation, (2) Gokak soil, a sandy loam free from wilt and (3) red soil where usually no cotton is grown.

TABLE XXVI.

Relation of wilt to the cotton crop grown in black, sandy and red soils.

	SERIES I			SERIES II			SERIES III		
Kind of soil	Plants grown	Plants wilted	Wilt percentage	Plants grown	Plants wilted	Wilt percentage	Plants grown	Plants wilted	Wilt percentage
Dharwar soil.	31	29	96·0	36	23	64·0	36	10	28·0
Gokak soil	39	32	82·0	33	20	60·0	34	18	53·0
Red soil	39	15	38·0	37	23	36·0	62	10	28·0

As seen from the results the Dharwar soils produce wilt abundantly under artificial conditions. The Gokak soils too do not lag behind in getting wilt-sick when the fungus is introduced in them. In the red soil it looks as if wilt is less but the results are not consistent. There does not, therefore, seem to be any difference with regard to wilt attack in these soils. Once they are infected with the fungus they seem to be as bad as the typical wilt-sick soils. Their freedom, therefore, from wilt apparently is due to the fact that the causal fungus has not been introduced into them.

When the general survey for wilt for the whole of the Bombay Presidency was made in the year 1928-29 a significant relation was apparent between the wilt disease and the type of soil. It has been found that the prevalence of wilt is confined to the black cotton soil which cover the whole of the Karnatak and the central division including Khandesh and extend as far as south Gujarat comprising the Surat district and part of the Broach district. It is totally absent in the

Goradu soil (sandy loams) of North Gujarat and the alluvial plains of the Indus in Sind. In the United States of America according to Young [1928] on the other hand wilt is found more frequently in the light sandy soils and is largely absent in the clayey soils.

That the reaction of the substratum is a very important factor in influencing the growth of many disease-producing organisms is a well-known fact. In order, therefore, to see whether the presence or absence of wilt in these soils has anything to do with their reaction, experiments were carried out to determine the pH values of these soils.

The method followed in determining the pH value was the colorimetric method. The values obtained are the average of five readings in each case. These readings were subsequently confirmed by the Agricultural Chemist to Government of Bombay, Poona, who used the potentiometer in determining the pH values.

The figures for the Sind soils were supplied by the Agricultural Chemist, Sind.

TABLE XXVII.

	Place	pH
Wilt tract	Dharwar wilt plot	7·8
	Dharwar non-wilt plot	7·8
	Gokak Farm soil (wilt-free tract)	7·9
	Akola Farm wilt plot	7·6
	Akola Farm non-wilt plot	7·6
	Jalgaon Farm wilt plot	8·0
	Jalgaon Farm non-wilt plot	8·0
Non-wilt tract	Goradu soil	8·0
	Sind soil	7·4 to 8·0

There is not much difference between the reaction of the soils in the wilt and in the non-wilt tracts and the reaction in all is within the range 7 to 8 at which the fungus grows well (Table XXVIII). The reaction of the soil therefore does not seem to be a controlling factor for wilt.

Influence af hydrogen ion concentration on the growth of the fungus.—For growing the fungus Richards' medium was used. The reaction of the medium was adjusted

by using normal hydrochloric acid or normal caustic soda. The range of initial pH values was from 3·2 to 9·7. The sterilisation always interfered with the pH value, and hydrogen ion determination was made in each case after sterilization. There were for each series three flasks containing 50 c.c. of the medium. They were inoculated with a spore suspension of the fungus in sterile distilled water, with one loopful for each flask and were incubated for 7 days at 28°C. Further growth was stopped after this period by adding concentrated hydrochloric acid and the mycelium was obtained by filtering through Gooch's crucibles. It was then washed several times before the constant weight was determined by drying in the steam oven. The pH values were determined colorimetrically.

TABLE XXVIII.

Influence of the pH of the medium on the growth of the fungus.

pH of the medium	Dry weight of the mycelium in m.m.
3·32	21·5
4·2	50·7
5·2	89·4
6·0	69·4
6·8	73·6
7·2	79·0
7·7	61·7
7·8	43·6
8·2	39·4
8·7	*Nil*
9·2	*Nil*

Good growth seems to occur over a wide range from 4·2 to 8·0. The best growth, however, is found at two points, one at 5·2 and the other at 7·2.

Organic matter.

The influence of organic matter in the soil on plant diseases that are caused by soil fungi has been noted by various investigators. Linford [1928] in the study of pea wilt observes " the character of a soil which appear to influence the disease most

significantly is its content of organic matter ". In the tobacco wilt study the experience of Johnson and Hartman [1919] does not seem to warrant the conclusion that an increased dose of organic matter favours disease. Fahmy [1928] states "that the amount of manure (organic) added has a direct influence on the infective capacity of the soil ". Dastur's [1929] observation that wilt is common in fields manured with poudrette or unrotted farmyard manure is in agreement with those of Fahmy [1928]. Fulton [1907] however reported the beneficial effect of stable manure in cotton wilt control. These conclusions except those of Johnson and Hartman are based on field observations or on scanty data. In order, therefore, to get experimental evidence on this question investigations were made both in pot culture and field plots. The general procedure to prepare the pots with soil for experiments was as mentioned on page 1003. The farm soil was mixed with various proportions of farmyard manure as noted in the table below, was sterilised in the autoclave and was then inoculated with the culture of the fungus grown in liquid medium. The control pots had sterilised soil without the fungus. There were four pots, two for treatment and two for control for each mixture of soil and manure. There were three series of the experiment and the results are tabulated below. Since there were no deaths due to wilt in the controls figures for them were not enumerated.

TABLE XXIX.

Influence of amount of organic matter on the cotton wilt.

Treatment	Duration of experiment 6th Aug. 1925 to 31st Oct. 1925			Duration of experiment 31st Oct. 1925 to 29th Dec. 1925			Duration of experiment 29th Dec. 1925 to 2nd Mar. 1926		
	Total plants	Deaths	Wilt percentage	Total plants	Deaths	Wilt percentage	Total plants	Deaths	Wilt percentage
Soil only . . .	28	28	100	38	38	100	35	30	85
Soil with manure									
90% „ 10% .	31	31	100	33	31	94	38	27	71
80% „ 20% .	26	25	96	38	21	55	37	24	66
60% „ 40% .	29	23	78	24	11	46	44	31	70
40% „ 60% .	27	17	63	37	15	40	47	24	51
20% „ 80% .	30	20	66	38	9	23	36	20	55
Manure only . .	35	24	68	34	8	23	34	9	26

As seen from the table the results in all three series clearly indicate that high percentages of organic matter reduce the number of deaths from wilt. The quantities of manure required to achieve this effect are larger than is practicable under field conditions, but it must be remembered that the plants were grown in soil which had been very heavily infected artificially and such high uniform wilt infestation are not generally obtained in field conditions, so that it was obvious that the question of the effect of the organic matter in reducing wilt loss should be followed up under field conditions. Field trials with a maximum dose possible in cultivation (40 tons per acre) were therefore tried. For the experiment two contiguous plots each with an area of 7 *gunthas*, were chosen from a field known for its wilt infestation. Seven tons of farmyard manure at the rate of 40 tons per acre were spread uniformly on the adjoining halves of these plots, the other two halves of each serving as controls. The manure was ploughed-in so as to get thoroughly mixed in the soil. The results of three years are given in Table XXX.

TABLE XXX.

Influence of farmyard manure on wilt in cotton crop grown in small plots in the heavily infested field.

Year	Plot	Treated			Control		
		Plants grown	Plants wilted	Wilt percentage	Plants grown	Plants wilted	Wilt percentage
1928-29 . .	I	715	324	45·3	726	267	36·7
„ „ . .	II	725	172	23·7	721	190	26·2
1929-30 . .	I	1,229	393	32·2	1,250	378	30·2
„ „ . .	II	1,204	423	35·1	1,285	478	38·0
1930-31 . .	I	1,310	338	25·6	1,255	370	29·5
„ „ . .	II	1,317	215	16·3	1,302	242	18·5

The results of plot I for 1928-29 and 1929-30 do not show the efficacy of the treatment, the deaths in the treated plot being more than in the control. In 1930-31 the deaths in the control exceed those in treated by about 4 per cent., indicating a good effect of the treatment. The results of the 2nd plot for all the three seasons are in favour of treatment. The difference between the percentages of treated and

control is not however very marked. But in noting the general trend of the disease month by month in the crop throughout the season for each year and on tabulating the results (Table XXXI and also graph in Fig. 4), it is evident that in treated plots a large number of deaths occurs in the first few weeks of the season when the plants are still in the seedling stage but wilt decreases in the latter half of the season when the plants are nearly mature. In the controls, deaths in the latter half are more than in the treated series. This fall of deaths in the treated plots in the latter half of the season is important. A loss of 25 per cent. at the seedling stage is not necessarily heavy but a loss of even 5 per cent. later on when the crop is nearly mature is certainly disastrous and irreparable. The results of farmyard manure thus seem to save this heavy loss in the crop in its later stage and might with advantage be applied not on a field scale but to treat small diseased patches of land.

TABLE XXXI.

Monthly death rates in cotton plants in plots treated with farmyard manure at 40 tons per acre for the years 1928-29, 1929-30 and 1930-31.

Month	1928-29		1929-30		1930-31	
	Treated	Control	Treated	Control	Treated	Control
August	2·2	2·3	8·3	7·1	2·2	1·5
September	8·1	5·4	16·64	11·3	13·1	13·9
October	5·9	4·9	5·13	7·5	4·8	7·2
November	2·16	2·2	1·72	3·0	0·4	0·5
December	5·4	5·8	0·5	1·2	0·3	0·4
January	5·9	7·0	0·62	3·8	0·4	0·3
February	1·18	4·2	0·0	0·08	0·1	0·4

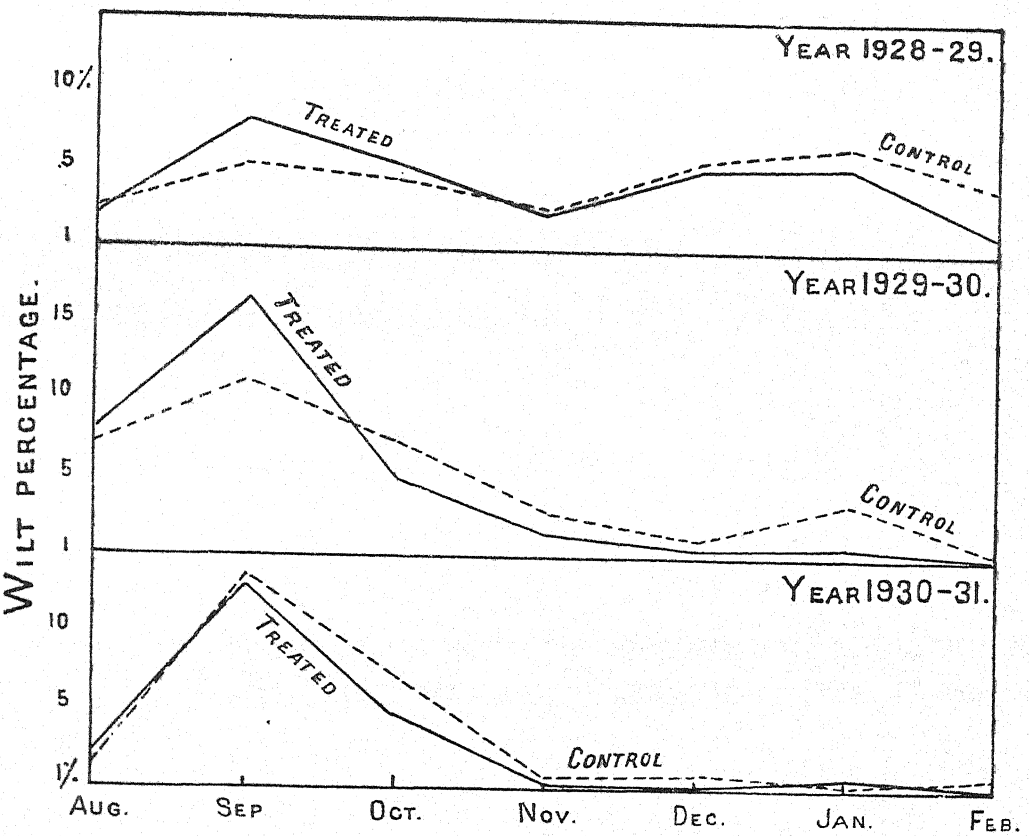


Fig. 4. The effect of farmyard manure in checking the disease in the cotton crop at its later stage.

Sowings at different periods in the season.

The object with which this study was started was to see how a change in the date of sowing the crop would operate on the severity of the disease. Three sowing dates were proposed, *viz.*, June, July and August (the last sowing being the normal for the tract). The early sowings did not have any effect in reducing wilt, but on the other hand they seem to increase it. This was in accordance with the known facts. Conditions for wilt production (as has already been noted) are most favourable in June and July because of the soil temperature at this period being at its optimum for the disease. The crop therefore when sown two and one months ahead of the usual sowing time gets its susceptible period for disease prolonged so as to expose the plants to all possible conditions of infection and consequently they die in greater numbers. The early sown (June and July) plants

however had a longer period for vegetative growth and consequently produced a larger number of flowers and bolls per plant as against the normal (August) sown ones and this had its effect on yield. Irrespective of the heavy attack of wilt they seem to give greater yield as seen from Table XXXII.

TABLE XXXII.

Observations on the sowings of cotton plants at different periods in the season regarding the incidence of wilt and yield.

Sowings	Years	Total plants grown	Plants wilted	Wilt percentage	Average yield per plant in grams	Yield per acre in lbs.
June	1928-29	622	172	27·9	40·0	731
July		543	112	19·6	25·4	521
August		614	75	10·5	14·8	396
June	1929-30	481	367	76·3	20·2	705
July		585	377	64·4	26·4	1123
August		526	331	62·8	11·9	453
June	1930-31	2449	96	3·9	16·6	625
July		2536	89	4·1	12·3	506
August		2649	58	2·2	10·8	447
June	1931-32	6789	1464	21·3	12·3	549
July		6498	1044	16·3	17·8	787
August		6734	977	14·3	15·6	611

During the first two years (1928-29 and 1929-30) there were no replications and for each sowing there were only five lines and the variety of cotton sown was Dharwar No. 1, the most susceptible to wilt. In the third year (1930-31) Jayawant cotton, a resistant variety, was tried with a view to avoid wilt trouble so as to get more reliable data on the yield question. There were three replications and each had nine lines. During the year (1931-32) the experiment was again repeated with

Jayawant cotton with six replications, each containing 12 lines. For yield calculations, only the middle 8 lines were considered, avoiding 2 lines at each side in order to minimise the experimental error due to the border effect. The results of these experiments suggested that cotton could with advantage be planted earlier than August in Dharwar tract.

Chapter V.

THE ROOT SYSTEM OF THE COTTON PLANT.

(*i*) *Root system in relation to disease.*

In the preceding chapter the influence of some of the soil factors on the development and expression of wilt disease in the cotton crop has been discussed. Now the root system of the plant will be examined with a view to see whether there is any correlation between it and the immunity, resistance or susceptibility of the plant to disease.

The idea of correlating the root system with the various aspects in crop production, such as growth, yield, disease resistance, has attracted the attention of scientists in recent years and a thorough knowledge of the root system and root activities has become recognised as *sine quanon* for raising plants for crop production. Indeed, in some of the cases studied failure of a crop in a locality has been traced to its root system being quite unsuited to the soil conditions. The economic significance of the study of root development in plants has been ably advocated by Howard [1917] and he has produced a considerable amount of evidence in support of his argument. In India, in fact, he was the pioneer in this field of investigation. It has been explored more exhaustively by Weaver [1926] in the United States of America in describing the root development of cereals, potatoes and fodder crops. But so far as any crop disease due to a definite organism is concerned, no data are available as regards the relation between the root habit of the plant and its susceptibility or resistance.

Method and material.—Root studies of three types of cotton (1) Dharwar 1 susceptible to wilt, (2) Dharwar 2, a resistant variety, and (3) Gadag 1, an immune type, were made. The first two types belonged to the *Gossypium herbaceum* group and the third was an American Upland type belonging to the *Gossypium hirsutum* group. All these types were unit selections and therefore had fixed characters with regard to their respective root habit.

The method used in excavating the roots was to dig a trench of required depth and of convenient width by the side of the plant so as to expose the tap and the

side roots. These were then washed out by spraying away the soil with a bucket spray pump.

Due to the moist nature of the soil the roots of the seedlings were quickly washed out. In the case of larger plants it took two to three days to expose the roots completely. The work was more laborious with a mature plant and it took four to six days to expose the entire root system including even the tender, delicate roots. Pencil drawings of the entire root system were made to scale on graph paper simultaneously with the washing of the roots. The whole picture was made as far as possible in the natural position in the vertical plane. The drawings therefore represent the extent, position, branching and feeding system to a fair degree of accuracy *in situ*. The pencil drawings were then inked.

The plants to be washed were raised in a field of uniform conditions and it represented a typical soil for the cotton crop.

The first washings were with the seedlings 15 days old. In the table below are given some of the characteristic differences of each type.

TABLE XXXIII.

Type	Height of hypocotyl in inches	Depth of tap root in inches	Length of side roots in inches	No. of side roots in the first inch soil
Dharwar 1—susceptible . .	2	14 to 15	4 to 5	3 to 4
Dharwar 2—resistant . . .	2	15 to 17	4 to 6	4 to 5
Gadag 1—immune . . .	3	15 to 16	6 to 7	8 to 10

The number of plants washed in each case (1, 2 and 3) was 13, 10 and 10 respectively. The average height of the hypocotyl in the susceptible and resistant types is the same but it is greater by one inch in the immune type. There is not any characteristic difference in all the three types with regard to the depth to which the tap root descends. The side roots seem to grow longer in the resistant types as against those in the susceptible, while they are decidedly longer in the immune. The main difference seems to lie in the number of side roots put forth in the first inch of soil. The number is more in the resistant than in the susceptible and is considerably greater in the immune (Plate LXIX).

The second washings were done at the end of October when the plants had completed their vegetative growth. The plants had grown to a height of 18 to 24

inches with branches having flower buds in formation. This increased and vigorous growth of the plant was reflected in its root development. The tap root in both the susceptible and resistant types had grown to a depth of 20 to 25 inches. The side roots were also increasing in length to a distance of 18 to 24 inches and were giving off secondary roots. In the immune type the tap root seemed to be restricted in growth since it never went beyond 16 to 17 inches. The concentration of the side roots and their great expansion from 24 to 36 inches in the first two inches below the surface soil was particularly marked. The lower portion of the tap root was devoid of any prominent secondary roots and tapered to a slender point (Plates LXX, LXXI and LXXII).

The incidence of wilt disease is at its maximum during the period when the crop finishes its vegetative growth, more than 60 per cent. of the plants succumbing to it. Therefore if any correlation between the root habit and the resistance to disease is to be found it should be during this time of plant growth when disease is most severe. No further root washings of mature plants were therefore undertaken.

Discussion.—Howard [1917] has shown that excessive soil moisture and consequent lack of aeration are the most important factors affecting root development under field conditions.

Though the development of the root system is governed by conditions similar to those that hasten or retard growth in general, soil moisture and aeration seem to be the most important limiting factors. Howard has stressed this point in explaining some of the crop maladies, especially the linseed wilt. The deep rooted linseed crop when grown in moist soil gets its lower roots killed due to asphyxiation and consequently the wilt results. So far as Dharwar conditions go, excessive soil moisture or water-logging is not a factor to be considered as has already been pointed out in the previous chapter. Nor is there any indication of this point being reflected in the root habit of suspectible or resistant varieties of cotton. In both these types there is so much similarity in the general system of root development as regards the tap root and its depth, the general distribution of side roots and their extent of length and branching, as not to indicate any significant difference. The immune type however differs. It has a shallow tap root which sends out lateral branches along the surface and a greater number of these are mainly formed within the first few inches of soil (Plate LXXII). It may be noted however that unlike the susceptible and resistant types which belong to same species (*Gossypium herbaceum*), the immune type belongs to a different species (*Gossypium hirsutum*) and consequently it has its own root habit. As to how its peculiar root system makes it immune from the disease is a question since its immunity is not a mere

escape from the disease due to its characteristic root habit which is mostly spread in the upper layers of soil where the wilt fungus is found most abundantly.

The study of the root development of cotton then does not indicate any clear-cut differences between the susceptible and resistant types. It however gives a general idea of the morphological characters and the activities of the feeding systems of the three varieties, *viz.*, Dharwar No. 1 (susceptible), Dhawar No. 2 (resistant) and Gadag No. 1 (immune) as obtained under the local conditions.

(2). *Root system in relation to environment.*

Having failed to find in the cotton plant any correlation between its root system and susceptibility or resistance to disease, study was devoted to work out in detail the general nature of the root habit of the plant and its response to various changes in the soil. The type of the plant chosen was Dharwar No. 1, a selection from among the local cottons. Washings were done at four different stages of the crop growth, *viz.*, (1) seedling stage, (2) completion of the vegetative growth, (3) flowering stage and (4) boll-bearing stage. Thus a continuous record of the development of the entire root system of the plant has been obtained. In the seedling stage the number of plants washed was ten and this number decreased as the plants developed and in the last stage it fell down to three only.

Seedling stage.—Washings were done on plants of one month old. There were only cotyledonary leaves during the first half of the month but by the end of the month new leaves and vegetative branches had appeared. The tap root had penetrated to a depth of 19·4 inches with an average development of 14·9. The secondary roots had extended laterally to a distance of 10 to 17 inches and the tertiary ones had commenced to grow. The course of side branches on the tap root was within the first 4 to 6 inches and was entirely parallel to the surface and consequently their activities were confined to the upper zones of soil (Plate LXXIII).

Vegetative stage.—The plants were 1½ to 3 months old. They were in a vigorously growing condition with a good many vegetative branches. The main stem had attained a height of 2 to 3 feet. The tap root had penetrated to a depth of 3½ to 4 feet and had reached the sub-soil. The secondary roots had a good many tertiary and quaternary branches and had gone to a lateral distance of 3½ to 4 feet with a tendency to bend downwards at their tip and the feeding roots were found in the deeper layer of the soil (Plate LXXIV).

Flowering stage.—During this period the plants were getting well fixed, all factors favouring their growth and development. The tap roots were steadily progressing in the sub-soil with feeders at the extremities. The secondary roots

had taken a definite downward course. The feeding activity was more localised to the deeper zones of the soil mostly, but whenever there were showers, the surface roots always showed reinduced activity by putting forth new rootlets (Plate LXXV).

Boll-bearing stage.—The plants had attained their full height and the root development had reached its last stage, the tap and the secondary roots being active in the sub-soil only. In certain cases the tap roots and the secondaries had penetrated the sub-soil for more than a foot, thus attaining a total depth of 5 to 6 feet. Their branching was quite remarkable for their zigzag course and had a sort of distorted appearance. The feeding roots were entirely absent from the upper zones of the soil and confined to the sub-soil layers (Plate LXXVI).

Discussion.—It is evident that in the seedling stage the root system is tender, mainly consisting of the tap root with a few horizontal side roots which are within four to five inches from the surface soil. There being sufficient moisture in the soil, the root activity is confined to the upper layers of the soil. In the second stage of plant growth, when the moisture in the surface layers of soil decreases, the root activity of the plant is pushed on to the deeper layers. Consequently, the tap root increases in length, reaching the sub-soil. The laterals too after extending sufficiently laterally branch profusely and begin to go down in search of moisture. In the flowering stage the plant attains the full growth. The increase of transpiration and the large amount of food material required for the formation of flowers and fruits necessitates the production of a large, vigorous, widely branched root system so as to get sufficient water from the deeper layers of the soil. In the boll-bearing stage the absence of moisture from the upper layers of the soil causes the plant to send its roots to the still deeper sub-soil layers. The branches coming off from the upper portion of the tap root, after horizontal extension, go downwards more rapidly than the main root and consequently several of them were nearly as long as the tap root with extensive feeding roots at their tips.

Chapter VI.

Test of wilt resistance of different cotton varieties.

Resistance to Fusarium wilt.—For the control of wilt diseases caused by the vascular Fusaria which are soil inhabiting fungi pathological investigations have not so far suggested convenient or effective remedies such as dusting, spraying, seed-treatment, which have been ordinarily used against other fungus diseases. In such cases a discovery or development through selection or breeding of plants resistant to disease has been found to be the only method of control. Despite the fact that the task of obtaining resistant or immune types is a laborious and arduous one, the efforts of workers engaged in combating many of these maladies of crops such as water-melon, cabbage, flax, cotton and others, have been endowed

with notable success. Resistant strains are now being used extensively for these crops in places where the disease is very destructive. In the cotton wilt investigation, therefore, this phase of the question, *viz.*, the testing of resistant varieties, has been an important item.

Literature review.

Among the workers that have endeavoured to produce wilt resistant strains of plants, Orton's name [1908] stands foremost. He was the first to tackle the problem successfully and has produced resistant strains in crops like water-melon, cowpea, and cotton. His chief work lay in developing a method by which maximum wilt infestation could be produced in field conditions. This he did by mixing in the soil, known to be highly wilt-sick, a large quantity of material affected with the disease together with the addition of sufficient material of pure cultures of the fungus. He grew his plants on such highly infested soil and made his selections from those survivals that stood such rigorous tests. As a result of several years' work he was able to produce two varieties of cotton, Dillon and Dixie highly resistant to wilt. Many of the varieties that are now being used in the cotton tracts of U. S. A. owe their origin to these two varieties.

Young [1932] has been able to evolve resistant strains suitable for the areas growing long and short staple cottons in Arkansas. The varieties mentioned by him are Dixie Triumph (Watson), Dixie Triumph (Marett), Dixie 14, Lightening, Express and Miller. His method of testing cottons was to grow them in a field artificially infected with the cotton wilt organism.

Fahmy [1931] claims to have produced strains highly resistant to wilt under Egyptian conditions. He grew his plants in their seedling stage in a greenhouse in pots using soil from wilt infested fields. After two months' growth the survivals were transferred to the wilt-sick fields for further test. By such treatment he was able to minimise the chance of any plant not being reached by the fungus.

Youngman [1930] in the Central Provinces has met the situation by isolating a strain of Verum cotton from local cottons, known as Verum 262. It resists wilt up to 90 per cent. as against the local cotton whose resistance is not more than 10 to 20 per cent.

Method of experimentation.

In conducting the field tests for resistance during the last ten years certain conditions have been noted as regards the influence they have on the incidence of wilt.

(1) The influence of soil temperature upon the development and progress of wilt has already been noted. The optimum, for wilt, ranges from 20°C. to 25°C.

(2) Under Dharwar conditions cotton is generally sown in the middle of August when the soil temperature is favourable for wilt development, and this favourable temperature continues till the end of September. Thus during this period of six weeks (middle of August to end of September) most of the plants in the crop die of wilt. But it has been found (*vide* Fig. 2 under Chapter IV) that soil temperature favourable to wilt development begins to prevail much earlier than August, *i.e.*, June-July when cotton sowing is possible. The earlier sowings (June-July) lengthen the period of susceptibility from six weeks to ten-twelve weeks and expose the plants to all possible conditions of infection and consequently the chances of susceptible plants escaping infection and death are minimised. It is obvious that soil temperature and time of planting greatly influence the development of wilt in the crop.

The wilt plot.—The tests for resistance were made in a special plot located at the Dharwar Experimental Station. Trials done subsequently under controlled conditions are discussed in the second part of this chapter. This plot was used previously over a period of years by the Cotton Breeder, Dharwar, for selecting and breeding his varieties for wilt resistance. It was chosen by him because of the high infestation of the soil with the wilt disease. In 1923 when our trials were started the plot received the following treatment:—A large quantity of diseased plant material (wilted plants chopped and made into powder) together with a heavy dose of fungus culture (grown in liquid medium) was added and was well mixed in the soil. By repeated sowings of cotton in the subsequent years the plot has become as highly and uniformly infested with the wilt organism as is possible under field conditions.

Field tests.—The varities each season included commercial types as well as selections which were developed by the Cotton Breeders at the different experimental stations. For purposes of comparison a susceptible variety (Dharwar No. 1) was sown at frequent intervals throughout the plot. Inspection of wilt attack was made once a week soon after the disease started. The disease might manifest itself in a variety of ways. Some plants are killed within two weeks after planting. Others persist a few weeks more. Some are stunted and have their fruiting branches killed and are consequently barren while a few go on till the end of the season behaving like normal plants and producing bolls, but show definite evidence of attack in their internal parts, such as the stem and roots. For the purpose of noting the wilt percentage in each variety all the above variations of attack are included. In order to eliminate the effects of variation from season to season and of variation in the time of planting, tests have been done on most of the varieties for more than five years. The method has, however, never given us varieties completely free from susceptible plants.

We have at present under cultivation (Appendix) a large number of strains collected from various places within and outside the Bombay Presidency. All the strains, including many of the improved types isolated from Kumpta (Dharwar No. 1 for example) and crosses of these with *Neglectum roseum* (Dharwar No. 1 × Rosea), remain highly susceptible. So also are the Banilla (one of the improved types of Khandesh) and Goghari A. 26 of Gujarat. Dharwar 1 tried for the last ten years (1923 to 1932), has a range of wilt attack from 32·53 per cent. in the year 1929-30 to 94·6 per cent. in the year 1931-32. The cross (Dharwar No. 1 × Rosea) gives a variation of 50 to 70 per cent. wilt. Banilla shows 47 to 80 per cent. and Goghari from 31 to 62 per cent. But it is satisfactory to note that some strains isolated from Kumpta (Dharwar 2 and Kumpta wilt resistant) are highly resistant, each having an average wilt per cent. of 5·2 and 2·75 respectively over a period of ten years. Among the types from other parts of India, a selection from Wagale (a Burmese cotton) has also been found to be resistant. It has an average of 2·95 per cent. susceptibility. Comilla 4-2, a new selection from Assam cotton, is another highly resistant type. Verum 262, a resistant strain bred in the Central Provinces, maintains its resistance under Dharwar conditions. Its wilt percentage has not gone beyond 8·6. Broach Deshi 8, a selection made by the Cotton Breeder, Surat, is another important addition to our resistant types. Jayawant, a cross made by the Cotton Breeder, Dharwar, between Dharwar No. 1 (a most desirable type for trade but susceptible to wilt) and Dharwar No. 2 (less prolific but highly resistant to wilt) has also inherited the wilt resistant capacity of the second-named parent. Its behaviour for the last three years has given quite uniform results with an average wilt percentage of 6 only.

The immunity of exotic cottons (*hirsutum* types) both acclimatised, such as Dharwar-American, Upland and Gadag No. 1, and newly introduced ones (Cleveland) and also of Egyptian cotton, is established.

In general, it may be said that Asiatic cottons (so far the types tried at Dharwar are from India, Burma, Siam, China, Turkestan and Persia) are susceptible and the cottons of the United States of America and Egypt are immune. The cotton wilt fungus is not known to attack any other crop plant.

Discussion.

The results of trials of cotton varieties, some of which have been tested for nearly ten years, indicate that there are differences in resistance within the cotton varieties and that through selection of individual plants that survive on the infested land, resistant types could be developed. The work of the Bombay

Agricultural Department has been continued along this line and has resulted in the successful development of varieties of cotton, such as Jayawant, Dharwar No. 2, Kumpta wilt resistant, etc., that could be grown in the wilt affected areas.

These varieties, though they maintain their resistance under normal field conditions, succumb promptly in pot-culture under optimum conditions for wilt production as seen from Table XXXIV.

TABLE XXXIV.

Serial No.	Variety	Field conditions			Controlled conditions			Plants remained healthy
		Total plants	Plants wilted	Wilt per-centage	Total plants	Plants wilted	Wilt per-centage	
1	Dharwar 2 . . .	188	25	13·2	276	267	96·7	9
2	Jayawant	178	18	10·3	291	277	95·2	14
3	Wagale . . .	187	9	4·8	320	318	99·4	2
4	Comilla . . .	180	3	1·5	328	322	98.2	6
5	Kumpta wilt resistant .	180	6	3·6	301	264	87·7	37
6	Verum 262 . . .	182	25	13·2	311	304	97·7	7

It appears, therefore, that the resistance in these cottons is stable under a certain set of conditions only. If these conditions alter their resistance breaks down. In other words, these cottons, though pure (homozygous) for other characters like form of leaf. boll shape, staple, etc., are impure for wilt resistance. It means that each type of cotton is a mixture of strains varying in resistance. Under certain conditions some resistant strains die and others remain unaffected. But these also get diseased when exposed to still more severe conditions. This assumption has been borne out by field trials of Jayawant cotton (as noted in Table XXXII, page 1028) where the June and July plantings have produced more wilt in the crop than the August planting because of the favourable conditions for wilt development, extending over longer period.

This raises the question as to what extent reliance could be placed on these resistant cottons. Will they maintain their resistance ? They should, if they are pure (homozygous) for wilt resistance. But their performance both in field and controlled conditions does not warrant the belief that they will. This irregular behaviour could be explained on the supposition that though these cottons are unit selections maintained for a number of years under selfed conditions, selection for

resistance has been made under field conditions where irregular infestation of the soil by the parasite and the fluctuations of the climatic conditions prevent a thorough and severe testing for resistance and consequently elimination of all the susceptible strains has not been achieved. Under controlled conditions, chances for their escape are very remote and only the resistant ones will survive as has been already noted in Table XXXIV. It may also be stated that even Dharwar No. 1 cotton, which is ordinarily most susceptible, seems to contain some plants highly resistant to wilt. Selection of such plants has yielded resistant progenies. Out of a large number of Dharwar No. 1 plants that were raised in pots under optimum conditions for wilt development in 1930, four plants survived and they were allowed to mature. The seeds of each plant which was selfed, were sown separately in the infected field with the following results :—

TABLE XXXV.

Date of sowing 12th August 1931.

Variety Dharwar No. 1	Total plants	Plants wilted
Plant No. 1	64	8
„ No. 2	12	4
„ No. 3	48	14
„ No. 4	63	27
Ordinary Dharwar No. 1 seed	181	170

It is, therefore, suggested that instead of making the selection for wilt resistance under field conditions the varieties should first be tested under controlled conditions in pot-culture and the survivals should then be transferred to field for propagation. The special technique to be followed for this method has already been described under Chapter IV.

At present in the breeding work that is carried on at Dharwar, mere selection has not been of much use since the resistant cottons are wanting in commercial qualities and consequently in order to combine resistance with other desirable characters crossing is being resorted to. As already seen the crosses so far got break down under experimental conditions both in field and pot-culture. Attempts should, therefore, be made to evolve plants resistant to wilt under most favourable conditions for the disease. It may be mentioned that in the case of cabbage yellows caused by *Fusarium conglutinans* Woll., Walker [1930] has been able to

isolate resistant varieties of cabbage which, when exposed to environmental conditions most favourable for the disease have remained perfectly healthy. In pea wilt caused by *Fusarium orthoceras* App. and Wr. var. *pisi*, Linford [1928] has demonstrated that resistance of pea varieties (Horal and Green Admiral) does not break down even under optimum conditions for wilt development. It is, therefore, likely that resistant cottons, if, on isolation they are found to be pure (homozygous) for wilt resistance, will maintain their character under controlled conditions too.

There is no evidence to show that resistant variety loses its resistance. Resistance is due to genetic factors and so long as the arrangement of these factors is maintained in the chromosome, the likelihood of its breakdown is very remote. Of course, like all other characters of plants, it might be influenced by environment to a certain extent. The other contingency, when its resistance might breakdown, is when it encounters a new (physiologic) strain of the fungus. This phase of the question will be discussed in the next chapter.

As already noted the task of obtaining plants resistant to disease is a laborious and arduous one and, therefore, the breeder is considerably handicapped in his attempts. First he must have a suitable type of land where conditions for disease development are optimum and uniform. Secondly, he shall have to contend against the vagaries of climate and seasons. It means that he is required to test the plants for some seasons so that the plants can encounter all possible fluctuations of environment. The technical method gives standard optimum infected conditions and obviates the difficulties of irregular distribution of the disease in the field conditions and cut short the process of testing plants for a long period of years. Plants could be raised in pot-culture under optimum conditions for disease development and survivals of such tests could be transplanted in the field for propagation. The whole process could be done in one year.

The method thus shows the possibility of building up pure (homozygous) types for wilt resistance or susceptibility which can form a basis for further work for breeding resistant cottons either by selection or hybridization.

Chapter VII.

Conclusions.

The following are the main conclusions that have been arrived at in the course of these investigations :—

1. The cause of the wilt disease of the cotton is a strain of the fungus *Fusarium vasinfectum* Atk. It belongs to the group of vascular parasites which are responsible for wilt diseases in cabbage, flax, tomato, water-melon and many other crops.

2. The cultural and nutrition study of the fungus does not indicate any marked decrease of the disease with the various soil treatments tried. Variations in the kind of soil, in moisture, in soil reaction are of too little use. Applications of organic matter (farmyard manure) in heavy doses of 40 tons per acre seem to control the disease in the later stages of the crop.

3. Temperature studies of the fungus show that it can tolerate a wide range from 20°C. to 40°C. with an optimum at about 28°C., and its thermal death point is beyond 50°C.

4. The fungus is found pretty deep (up to 20 inches) in the soil. The soil temperature below six inches does not rise above an average of 32°C. The fungus, therefore, once introduced can remain in the soil for a number of years. It has been known to survive for seven years in a field which was not sown with susceptible varieties of cotton during the period.

5. There appears to be a close connection, however, between the temperature of the soil and the virulence of the disease. The disease is very severe at temperatures between 20°C. and 27°C., decreases at 28°C. to 31°C. and is completely stopped at 32°C. and above. Field experiments based on the results of this study which involved hot weather cultivation, sowing on ridges as against the ordinary practice flats and change in the time of sowing, have not yielded satisfactory results.

6. The fungus has not been known to attack any crop but cotton.

7. It has been found out that the disease is carried inside the seed. The possibility of introduction of the wilt organism into the soil by way of seed has been proved.

The practical utility of the conclusions is as under :—

(*a*) Though the correlation established between the soil temperature and the development of disease cannot be made use of in devising control measures under field conditions, it is still an important step in answering some of the preliminary questions in relation to environment and the occurrence of cotton wilt. It has enabled us to ascertain the reason for its prevalence in the Karnatak, Khandesh and Berar and its absence in North Gujarat, Sind and the Punjab. From the practical point of view this discovery has been utilised in evolving a special technique for the production of wilt resistant cottons, which the breeder can adopt with considerable saving of time and expense. The method consists in growing plants in pot culture under optimum conditions for disease development for two months and transferring the survivals of such rigorous tests to the field for propagation.

(*b*) The temperature factor could also be utilised by the breeder in testing his resistant cottons under field conditions. In the Karnatak the sowing of cotton

begins from the middle of August. Conditions for wilt development, however, are found to be quite favourable from June to the end of September. The susceptible period of the crop to disease could therefore be prolonged by making the sowings earlier than August in order to expose the plants to all possible conditions of infection and consequently the chances of the susceptible types escaping wilt infection are considerably lessened.

(*c*) Early sowings (June and July) have given higher yields than normal sowing (August). Though this is a side issue of the wilt question it is an important contribution to the knowledge about the cotton crop in general. By varying the sowing date of the crop the yield could be appreciably increased.

(*d*) The possibility of the disease being carried in the seed having been conclusively established, the district authorities could now regulate the policy of seed distribution so as to avoid spread of the disease to new areas.

Recommendations for future investigations.

The lines on which the future wilt investigation should be carried on are the following :—

1. Physiologic specialization in cotton wilt Fusarium.—In the development of disease resistant varieties of crop plants one has to take into account not only the causal organism of the place where one is working but also its different strains or physiologic forms. Otherwise a resistant variety of a place bred to one form of parasite may succumb when grown in another place to another form of the fungus. The existence of physiologic forms in morphological species of pathogenic fungi has been proved in many cases. This is a common phenomenon in rusts, smuts, mildews and many other fungi. It has also been noticed in the Fusaria causing wilt disease of crops. Broadfoot [1926] mentions eight physiologic forms of *Fusarium lini* Bolley, causing wilt in flax. With the cotton wilt *Fusarium* no detailed studies have been carried out on this problem. Young's [1926] preliminary account seems to indicate that there are distinct strains in the cotton wilt Fusaria of the United States. Fahmy's work [1928, 1931] in Egypt, like that at Dharwar, has shown that the American cotton *Fusarium* does not attack Indian cottons and, *vice versâ*, the Indian *Fusarium* does not attack American cotton.

It is not known whether the Indian Fusaria of such different places as Dharwar, Jalgaon, Akola, Broach and Cawnpore are identical in respect of their parasitism and other characters. In attempting to develop cotton varieties resistant to wilt, it is essential to know these Fusaria with regard to their number, geographical distribution and pathogenic properties. Without such a study the development of resistant varieties will not be complete. As stated already, a variety bred in one

place and resistant to one form of the fungus may not be useful at another place where a different form of the fungus exists. In the same locality it might be resistant for certain years and might suddenly become susceptible because of the appearance of a new form of the fungus. It is therefore necessary to know the reaction of resistant varieties to all the physiologic froms known to exist at the places where these varieties are to be introduced. If this is not done, there may be considerable delay and disappointment in the development of resistant varieties.

2. Inheritance of disease resistance.—In breeding plants resistant to disease knowledge of inheritance of disease resistance is of considerable value. This is especially true in the cases where resistant plants are to be obtained by crossing with a view to combine the resistance of one plant with the desirable qualities of the other. The study of disease resistance on factorial lines puts the breeding programme on a satisfactory basis and helps to attain the desired end with considerable ease and quickness. The importance of this study, as emphasised in the following quotations, is worthy of attention.

"Knowledge of mechanism of inheritance of character is a sign-post marking the breeder's path. Much plant breeding is done purely by the trial and error method. This is necessarily so since the genetics of comparatively few specific characters in economic plants is known. When the genetics of the characters concerned is understood, as now in the case of pea wilt, the results of each step can be predicted in advance, and the synthetic plant breeding attains the exactness of a science with all the economies in time and labour which this implies."

Genetic study of disease resistance has shown that it is a heritable character and in most of the cases studied has been found to be controlled by a single factor.

Biffen [1905] found in the case of the wheat rust, *Puccinia glumarum*, that resistance was a simple recessive character. Melchers and Parker [1922] working with *Puccinia graminis tritici*, concluded that resistance was dominant. Walker [1930] has demonstrated that in 'Cabbage yellows' resistance is dominant and is governed by a single factor. Wadé [1929] has studied the inheritance of pea wilt showing that resistance behaves as a simple dominant character to susceptibility. Only in the case of the flax wilt Burnham and Brink [1930] indicate that resistance is controlled by more than one factor.

Very little work has been done on the genetics of cotton wilt. We do not know whether resistance is dominant or recessive, or the number of factors that control it. Fahmy [1931] has devoted some attention to this question. He classifies plants according to wilt reaction, as immune, resistant and susceptible, and from the study of some crosses of these up to 3rd generation he concludes

"That immunity to wilt disease seems to follow Mendelian segregation and the existence of intermediate types of varying capacity of resistance indicates the complexity of the combinations possible among the factors deciding whether the plant is to behave as immune, resistant or susceptible." In some crosses immunity was found to be dominant over susceptibility and segregation occurred in the ratio of 3 : 1. He further says "That the results, however, though suggestive are not precise enough to indicate the genetic composition of plants".

Our preliminary study at Dharwar of this question indicates that, broadly speaking, all the Indian cottons, on the basis of their reaction to wilt, fall into two groups, susceptible and resistant. In between these two groups come intermediates with varying degrees of resistance. But these, when tested under optimum conditions for wilt, succumb completely, showing that their partial resistance under field conditions is due to environment and not to heredity. Crosses between the different resistant and susceptible strains have been carried up to the first generation with the following results :—

TABLE XXXVI.

Variety			Plants grown	Plants wilted	Wilt percentage
Susceptible		Resistant			
Rosea	×	K. W. R.	114	114	100
Rosea	×	Comilla	70	15	21·3
Dharwar 1	×	K. W. R.	42	33	78·5
Dharwar 1	×	Comilla	56	12	21·4

In cases when K. W. R. is one of the parents, resistance was recessive, but when Comilla was one of the parents, it is dominant. It thus shows that different varities carry different factors for resistance. Since no study has been carried to further generations the interpretation of the results on factorial lines would be merely arbitrary. Nor can the purity of the parents with regard to susceptibility or resistance be vouchsafed.

The study should, therefore, be carried on using pure lines. It is only then possible to know whether resistance is dominant or recessive and also the number of factors that control it.

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Appendix

Statement showing the wilt percentage of cotton varieties tried for wilt resistance.

Serial No.	Variety	1923-24			1924-25			1925-26			1926-27			1927-28			1928-29			1929-30			1930-31			1931-32			Average for 9 years
		Total plants	Plants wilted	Wilt per cent.	Total plants	Plants wilted	Wilt per cent.	Total plants	Plants wilted	Wilt per cent.	Total plants	Plants wilted	Wilt per cent.	Total plants	Plants wilted	Wilt per cent.	Total plants	Plants wilted	Wilt per cent.	Total plants	Plants wilted	Wilt per cent.	Total plants	Plants wilted	Wilt per cent.	Total plants	Plants wilted	Wilt per cent.	
1	Dharwar 1	551	334	60·6	330	209	63·3	1457	912	62·6	708	378	53·4	676	520	77·9	1523	648	42·5	1073	349	32·5	509	174	34·2	867	820	94·6	57·8
2	Dharwar 2	490	33	6·7	337	9	2·7	326	15	4·6	203	9	4·4	151	6	3·9	238	4	1·6	205	14	6·7	173	5	2·9	188	25	13·2	5·2
3	$D_1 \times D_2$-F6 strain 1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	234	23	9·8	...	...	...	...	...	...	...	...	...	9·8
4	$D_1 \times D_2$-F6 strain 1-12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	242	11	4·5	...	...	...	...	...	...	...	...	...	4·5
5	$D_1 \times D_2$-1-12-7	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	109	15	7·5	...	...	...	...	...	...	7·5
6	$D_1 \times D_2$-6-7-3	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	204	13	6·4	...	...	...	...	...	...	6·4
7	D_1 ($D_1 \times D_2$-1-12-7)-F1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	20	5	25·0	...	...	...	...	...	...	25·0
8	D_1 (Kandgol)	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	79	11	13·9	...	...	...	13·9
9	Wagale	546	21	3·8	313	12	3·8	239	4	1·7	187	9	4·8	157	0	0·0	230	3	1·3	220	10	3·6	176	5	2·8	187	9	4·8	2·9
10	$D_1 \times$ W-F7-13-9-8-4	...	...	...	...	...	...	...	..	...	...	...	...	...	...	...	256	15	5·8	211	17	8·1	...	...	...	...	...	...	6·9
11	$D_1 \times$ Wagale-F7-13-9-8-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	230	14	5·8	245	19	7·7	...	...	...	...	...	...	6·7
12	$D_1 \times$ W-13-9-3-6-3-4	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	222	6	2·7	188	29	15·5	9·1
13	Comilla 4-2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	212	3	1·4	201	7	3·5	180	3	1·5	2·1
14	Comilla 6-1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	198	11	5·6	...	...	...	178	4	2·4	3·9
15	Comilla 5-3-3	...	...	...	...	...	..	...	...	...	...	...	...	...	...	...	...	...	...	223	56	25·1	...	...	...	...	...	...	25·1
16	Comilla 3-6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	213	6	2·8	...	...	...	...	...	...	2·8
17	Cutchicum	...	...	...	...	...	...	...	...	...	...	...	...	107	79	73·8	...	...	...	...	...	...	...	...	...	...	...	...	73·8
18	Cleveland big boll, susceptible to American wilt fungus.	...	...	...	...	...	...	...	...	...	74	0	0·0	118	0	0·0	...	...	...	...	...	...	...	...	...	...	...	...	0·0
19	Broach Desi 4	...	...	...	174	161	92·5	19	15	78·9	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	85·7
20	Broach Desi 6	...	...	...	136	44	32·4	75	29	38·6	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	35·5
21	Broach Desi 8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	199	13	6·3	58	3	5·2	195	9	4·6	172	12	7·1	5·8
22	Dixie wilt resistant	...	...	...	147	0	0·0	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	0·0
23	Dixie triumph resistant to American wilt fungus.	...	...	...	...	...	...	...	...	...	73	0	0·0	108	0	0·0	...	...	...	...	...	...	...	...	...	...	...	...	0·0
24	Kumpta wilt resistant	342	16	4·7	193	11	5·6	313	3	0·9	176	1	0·6	161	3	1·9	239	8	3·3	232	11	4·7	185	1	0·5	180	6	2·6	2·8
25	Kumpta-Dharwar	...	...	...	...	...	...	...	...	...	99	53	53·5	...	...	...	211	81	38·4	...	...	...	...	...	...	...	...	...	45·9
26	Kumpta-Hubli	...	...	...	195	52	26·6	...	...	...	118	72	61·0	...	...	...	227	88	38·7	...	...	...	...	...	...	...	...	...	42·9
27	Kumpta-Haveri	...	...	...	125	34	26·4	343	201	58·6	...	...	...	...	...	...	210	78	37·1	..	...	...	...	...	...	...	...	...	40·7
28	Kumpta-Gadag	...	...	...	122	60	49·2	...	...	...	178	91	57·1	...	...	...	221	97	43·9	...	...	...	...	...	...	...	...	...	50·0
29	Kumpta-Bailhongal	...	...	...	184	79	42·9	346	146	42·2	121	80	66·1	...	...	...	205	72	35·1	...	...	...	...	...	...	175	47	27·0	42·7
30	Kumpta-Bijapur	...	...	...	126	107	84·9	...	...	...	78	73	93·6	...	...	...	164	127	77·5	...	...	...	...	...	...	...	...	...	85·3
31	Kumpta-Bagalkot	...	...	...	147	89	60·5	...	...	...	143	107	79·8	...	...	...	206	137	66·5	...	...	...	...	...	...	...	...	..	68·9
32	Kumpta-Bagewadi	...	...	...	...	...	...	274	171	62·4	97	81	83·5	...	...	...	178	142	79·6	...	...	..	...	...	...	...	...	...	75·2
33	Kumpta Farm	497	50	10·1	222	20	9·0	392	114	29·0	133	63	47·3	179	23	12·8	...	...	...	199	4	2·0	178	7	3·9	182	8	4·3	14·8

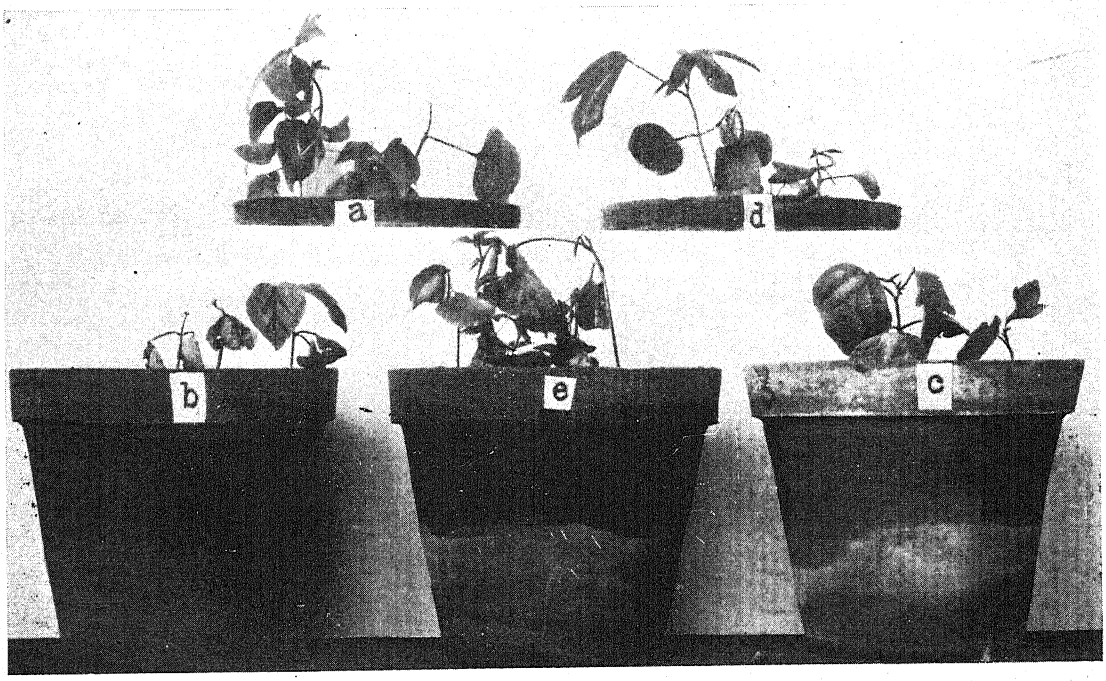


Fig. 1. Seedlings showing wilt infection, grown from seed collected from wilt affected cotton plants: *a*, *b* and *c* pots from Cawnpore seed, *d* and *e* pots from Jalgaon seed.

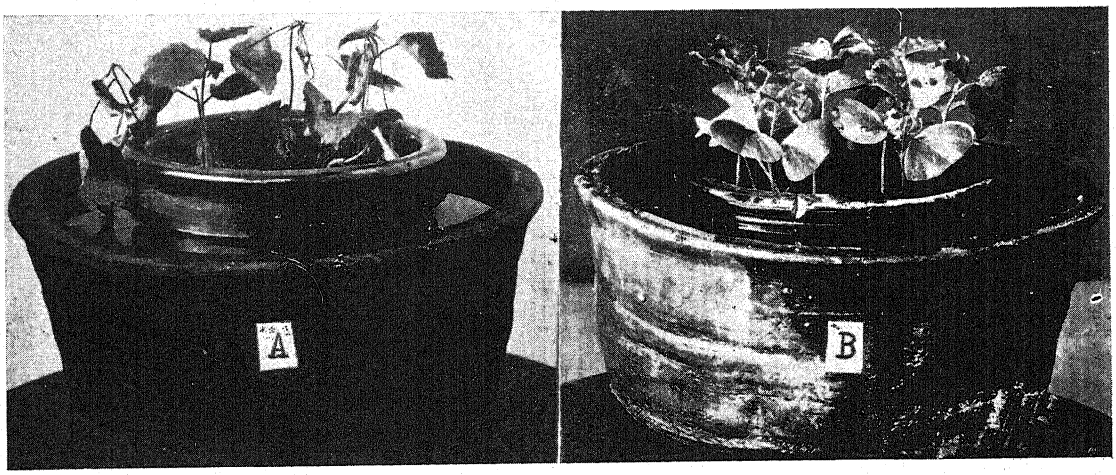
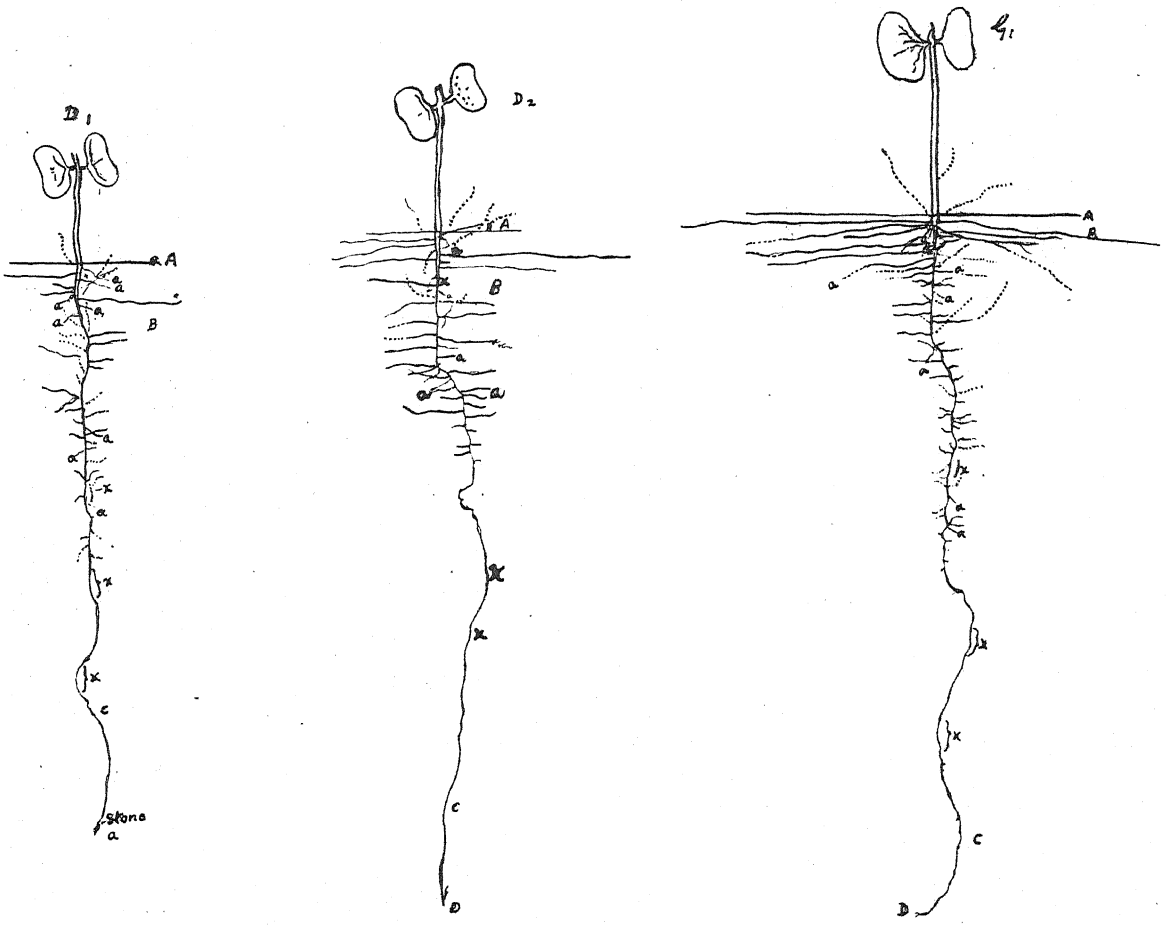


Fig. 2. Special technique used for growing plants at soil temperature lower than the air temperature: (*A*) Plants in infested soil, (*B*) Plants in uninfested soil.

ROOT-SYSTEM IN COTTON SEEDLINGS.



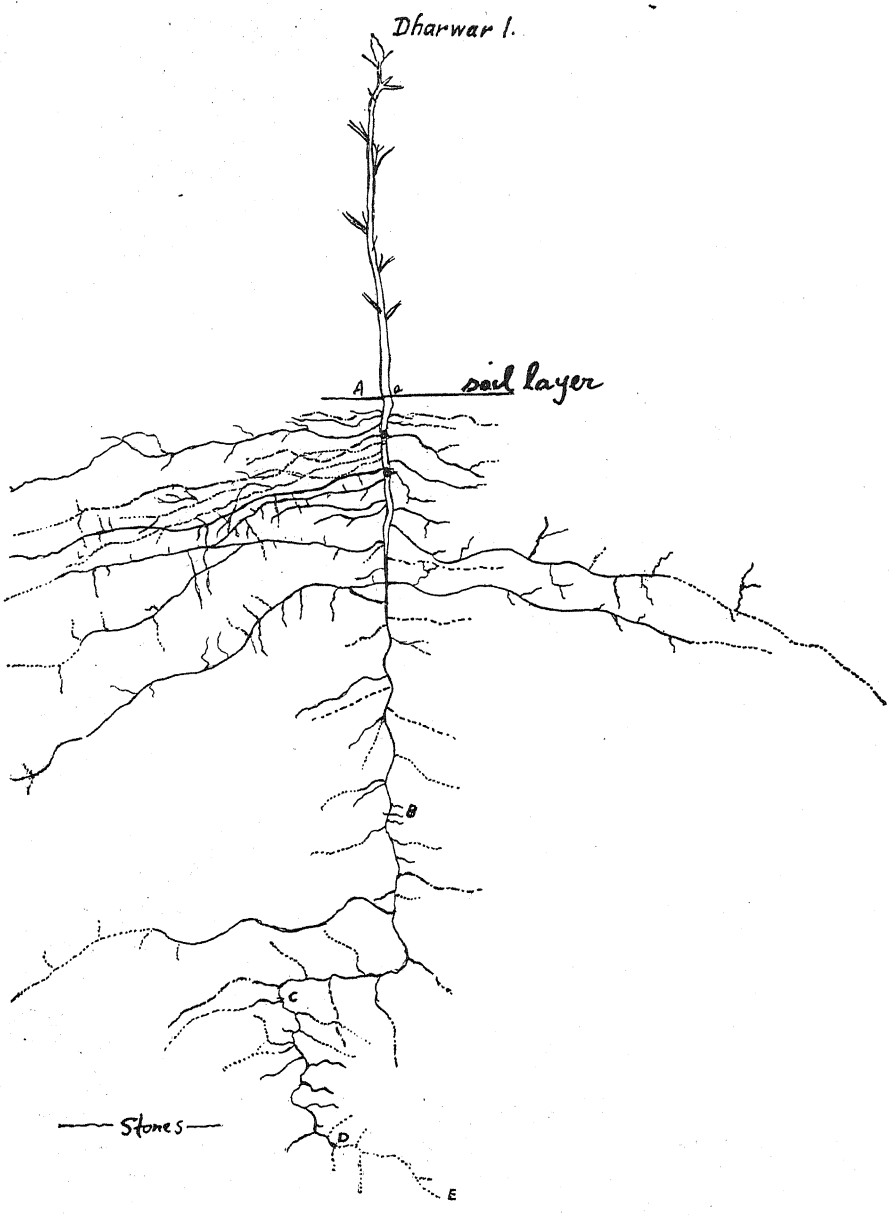
D_1. Susceptible. D_2. Resistant. G_1. Immune.

(Roots are quite tender in all varieties, they are thicker in G_1. Root hairs not visible.)

(*Date of sowing 18th August 1927 ; date of washing 28th August 1927.*)

A—B = yellowish.
B—C = dirty white.
C—D = pearly white.
X = yellow portions.
a = secondary roots in front.
..... secondary roots passing behind.

PLATE LXX.



Root development in D_1 type of cotton plants at the end of vegetative period.

Sown on the 18th August 1927. Examined on the 3rd October 1927.

A to B, deep yellow. B to C, yellowish. C to D, dirty white. D to E, white.

..... going behind. .—.—.—. going in front.

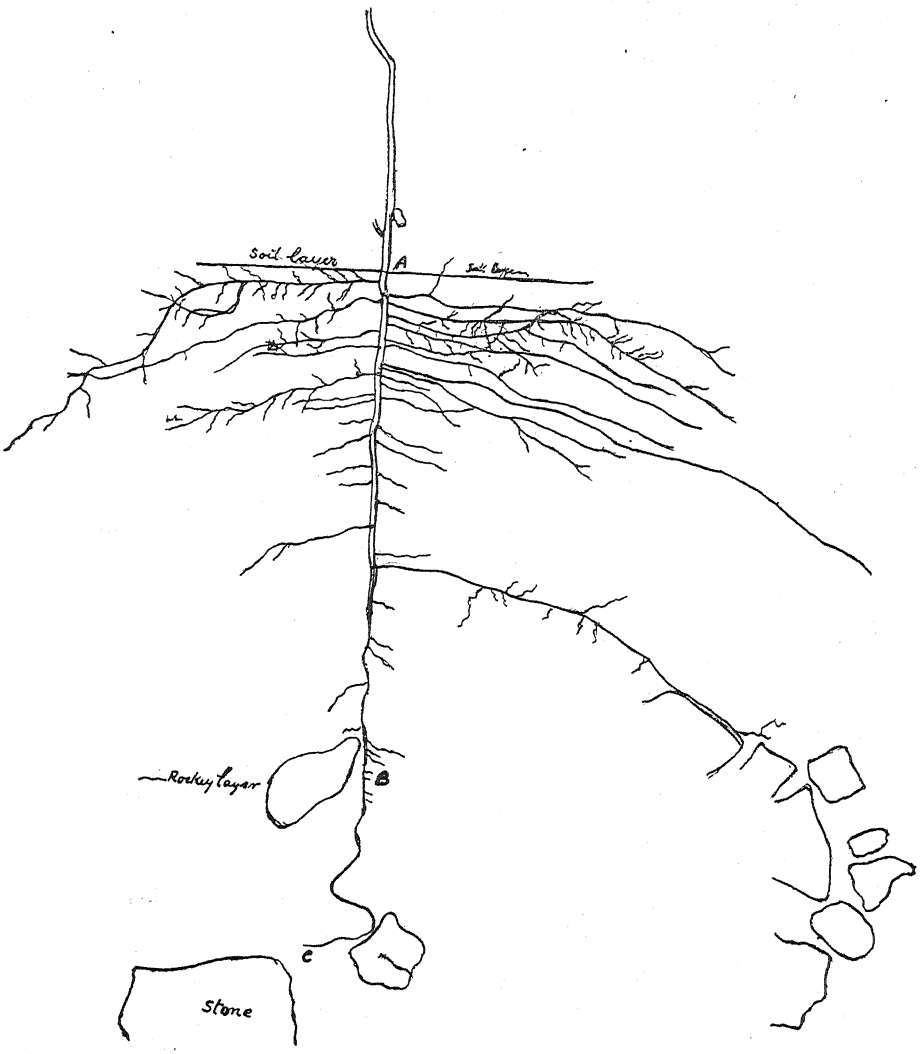
Height of the stem 10·0″. Branches ow. 2. Branches ax. 5. Branches Tr. 5. Tap root penetration 25″.
Depth of the soil 11″. Subsoil stones. Lateral range of the sec. roots 18″.
Vert. 8″. Tertiary 2·0″. Quat. 6″.

Note that the roots are going deeper down at this stage.

Note the scanty growth of the sec. in the 1st two inches (cf. D), their shorter length (cf. D. & G.) and less thickness (cf. G).

Quaternary are almost absent and the whole root system is not so profused as in D & G .

Dharwar 2.

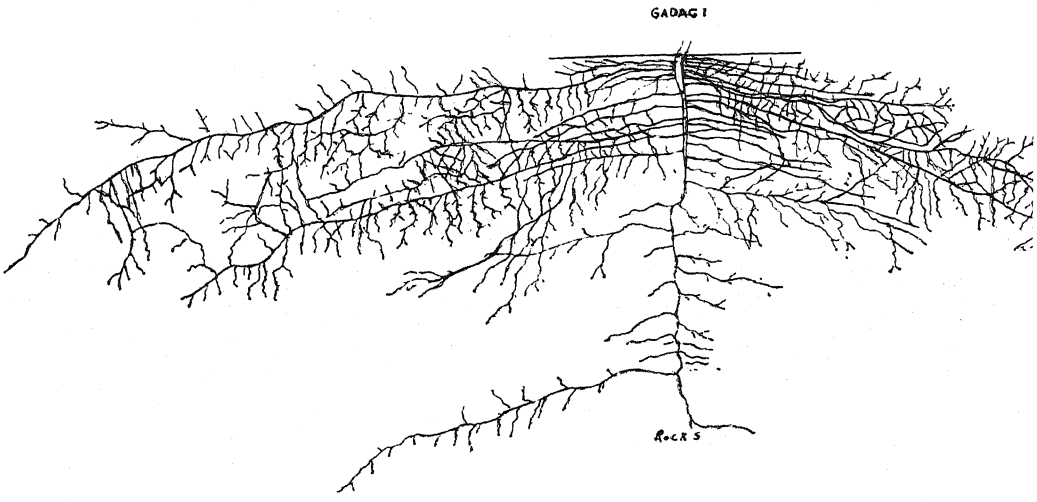


Root development in D_2 type of cotton plants at the end of vegetative period.
Sown on 18th August 1927. Washed on 1st October 1927.
A to B, deep yellow. B to C, yellowish. C to D, white.

Height of the stem 8·2″. Branches ow. 3. Branches ax. 2. Branches Tr. 4. Tap root penetration 25·2″. Soil depth 16″. Sub soil Rocky. Lateral extent 22″. Vert. extent 11″. Tertiary extent 4″. Quat. extent 5″.

Note that the secondary roots are now rapidly going deeper.
Note the wavy appearance of the tap in the rocks.
No concentration of the roots in any zone (cf. G_1).
Tert. and Quater. are rapidly appearing.

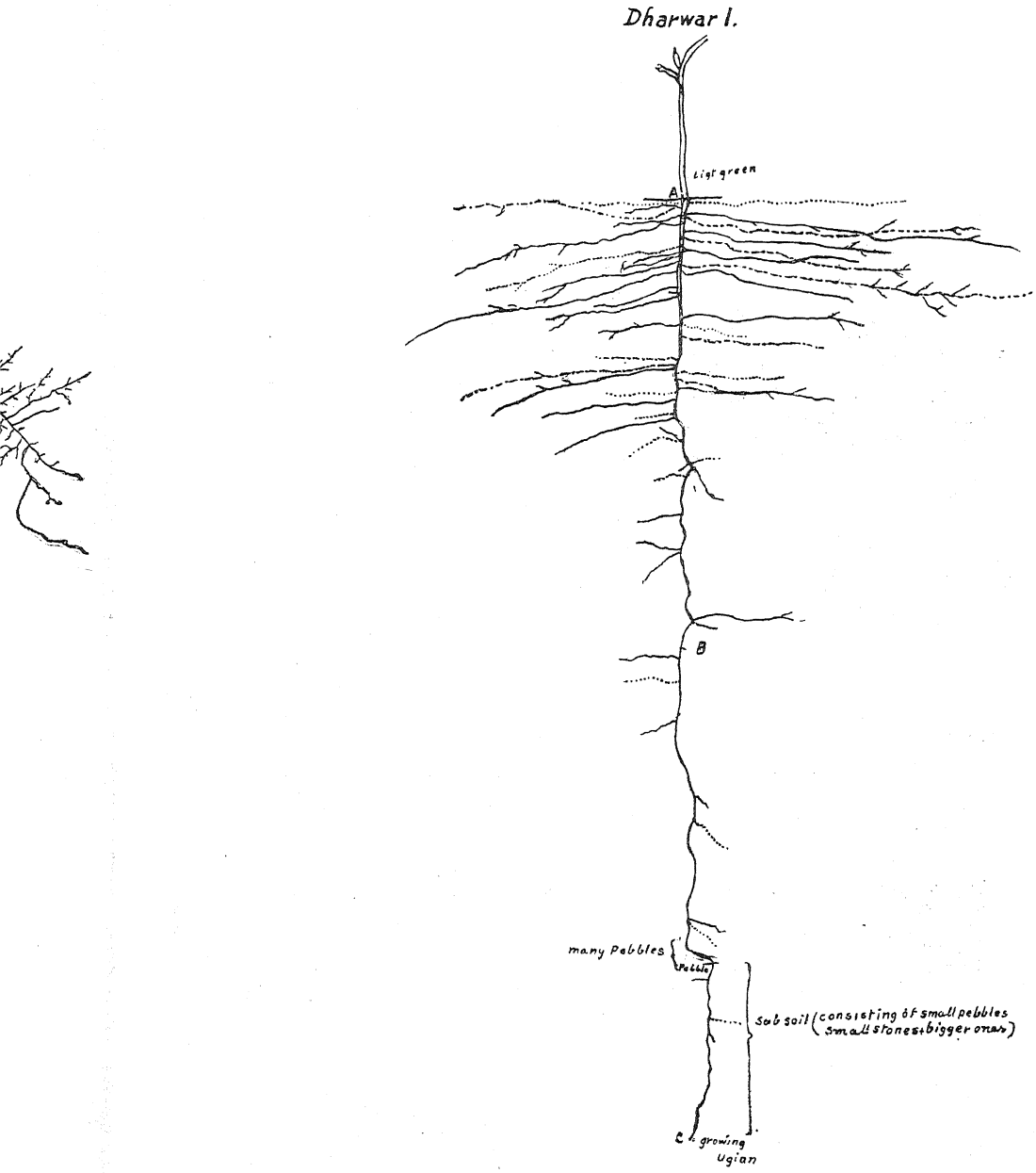
PLATE LXXII.



Root development in G_2 type of cotton plants at the end of vegetative period.

Sowing on the 18th August 1927. Washing on the 13th October 1927.

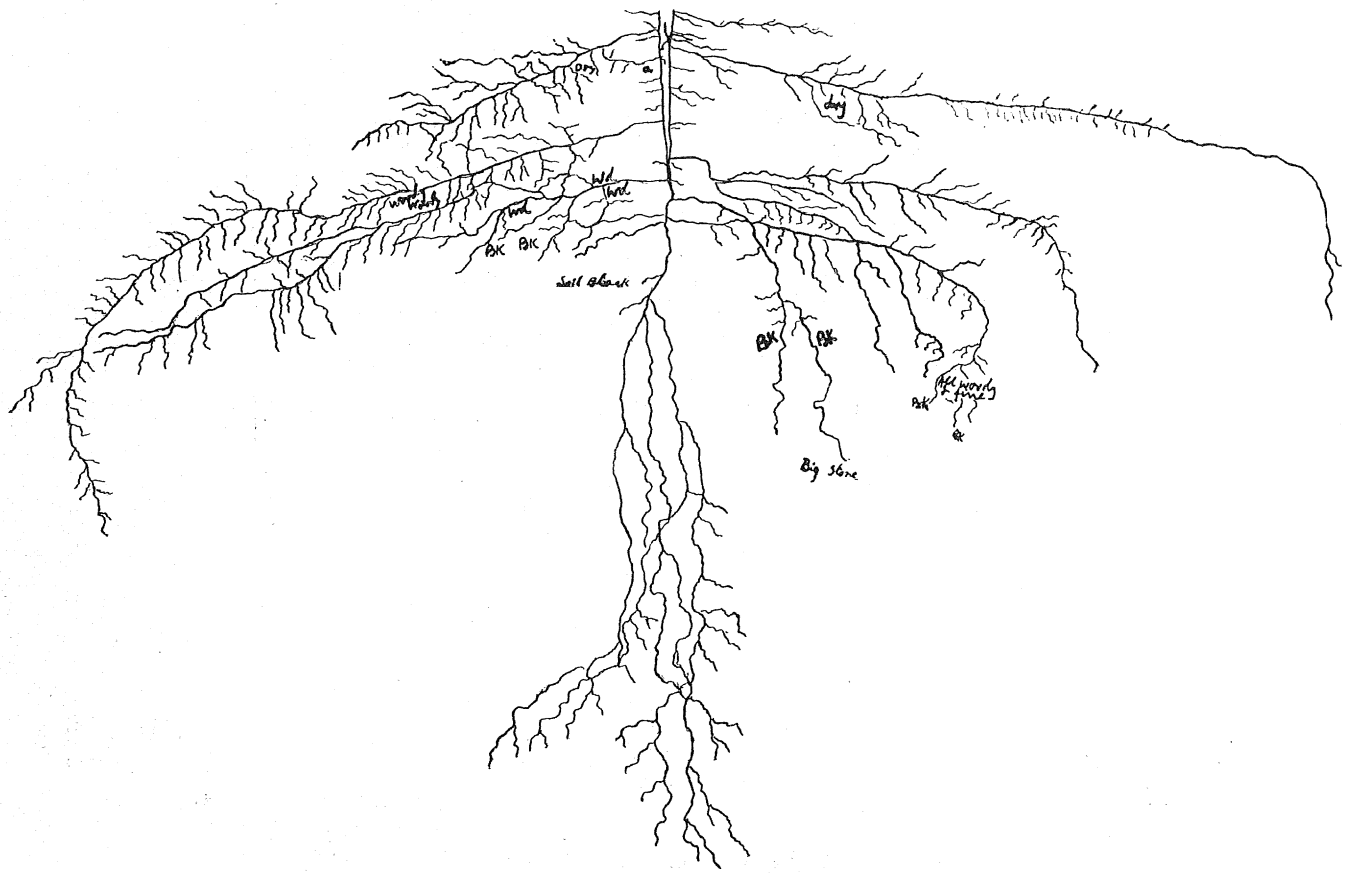
Stem 14 inches.
Branches ow. 2
,, ax. 3
,, Tr. 5
Tap root penetration—18″ (could not be traced further because of rocks).
Soil depth 18″.
Lateral range of the Sec. roots 38″ & 41″.
Tertiary — 15″.
Quat. — 4″.



Root development in Dharwar No. 1 cotton in seedling stage.

Sown on the 18th August 1927. Examination on the 7th September 1927.

Height of the stem 2·3″. Branches ordinary 2. Tap root penetration 16″. Depth of the soil 14·4″.
Sub-soil — Pebbles and stones. Lateral range of the sec. roots 6″. Tertiary 6″.

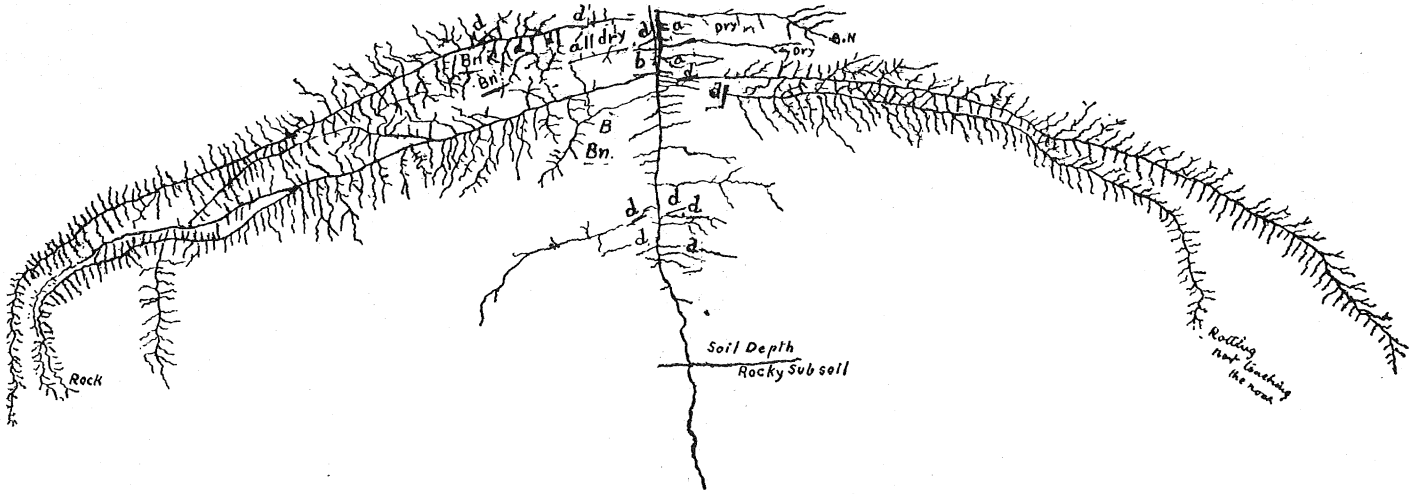


Root development in Dharwar No. 1 cotton (flowering stage).

Sowing date 18th August 1927.

Stem :— { Height 31″
Branches { Ord. 3 | Axil. 6 | Fruit. 13

Depth of soil 16″. Sub-soil : A hard layer of lime (which is moister compared with the upper layer of black soil). Nodules with big stones here and there. Penetration of tap root 48″. Horz. range of secondary roots 37″. Vert. range of secondary roots reach the sub-soil. Degree of branching, Quaternary (penternary rare). In the upper six inches soil was cracked and very dry. Feeding roots practically absent in the first six inches and very few in the second six inches layer also. The feeding roots in the sub-soil are thicker and more suckulant.



Root development in Dharwar No. 1 cotton (vegetative stage).

Dharwar 1 Wilted partially.

Date of sowing 18th August 1927. Date of examination 29th October 1927.

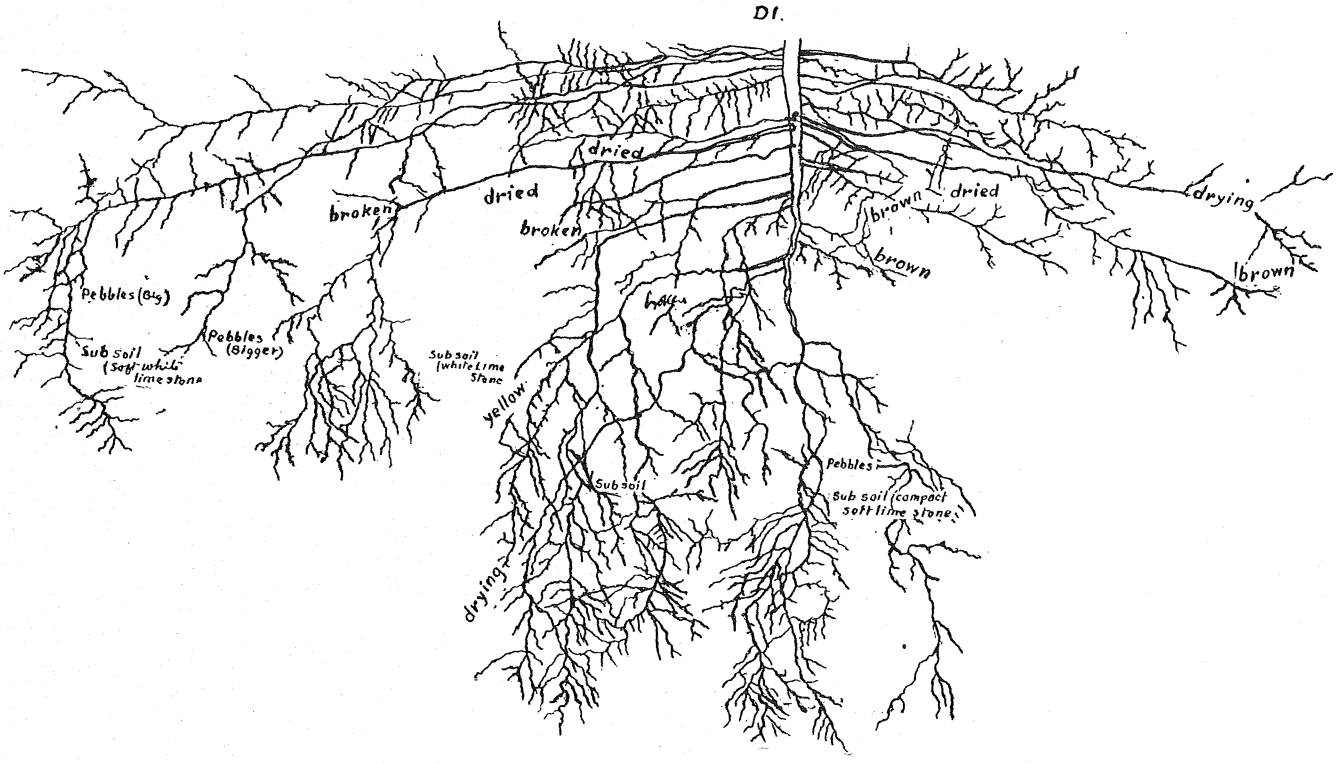
Quaternary roots are present. Penetration of the tap root 28·4. Horizontal range of the secondary roots 44″. Vertical range of the secondary roots reach the sub-soil. d :—drying (Note most of the feeding roots in the first six inches are drying) Br :—Brown discolouration. a :—roots coming in front. b :—going behind.

Stem :— Height 20″

Branches	Ord.	Axil.	Fruit.
	3	4	10

Depth of soil : 21″. Sub-soil :—Rocky (bare rock). Tap root had entered a crevice but was successfully removed up to the growing point. Tertiary on an average 2″ to 6″ (only in two cases they are greater in length). Quaternary : 2″ to 3″. Penternary not present. Roots when split open show the typical black discolouration.
Feeding roots in the lower depth are thicker and healthier.

PLATE LXXV.



Root development in Dharwar No. 1 cotton (boll-bearing stage).

Sown on the 18th August 1927. Examined on 17th December 1927.

Height of the stem 54″. Branches ow. 3. Branches ax. 10. Branches Tr. 21. Tap root penetration 44″. Depth of the soil 26″. Sub-soil consists of compact lime and shell. Lateral range of sec. roots 46″. Vert. range of sec. roots 16″. Tertiary range of sec. roots 16″. Quat. range of sec. roots 8″. Pent. range of sec. roots 2″.

Most of the side-roots which had appeared as a result of the rains are now drying. The side roots have penetrated down to a great depth. Lateral growth is coming to a stand still.

A NOTE ON THE REAPPEARANCE OF WATER HYACINTH SEEDLINGS IN CLEARED TANKS.

BY

P. PARIJA, M.A., I.E.S.,

Professor of Botany, Ravenshaw College, Cuttack.

(Received for publication on 5th October 1934.)

In the final report on the water hyacinth which was published this year, I had concluded from field observation [Parija, 1934] that the seeds of water hyacinth retain their viability for 5 years at least. Tanks cleared of water hyacinth in 1927 put forth seedlings in 1932. This year I visited one of these tanks on the 28th June and found a number of seedlings at various stages of development. On enquiry it was found that the tank had completely dried up this summer and the bottom was thus exposed. After the first shower of rain seedlings appeared. The tank has been kept free from adult water hyacinth for the last 7 years and thus there was no chance of fresh seeds being added to the tank. This shows that the seeds which had fallen to the bottom of the tank prior to 1927 had retained their viability for 7 years at least.

REFERENCE.

Parija, P. (1934). *Ind J. Agric. Sci* **4**, 401.

CHROMOSOME NUMBER IN SUGARCANE × SORGHUM-HYBRIDS

BY

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Botany Assistant, Imperial Sugarcane Station, Coimbatore.

(Received for publication on 11th October 1934.)

(With Plate LXXVII)

Dr. G. Bremer [1923] of Java was the first to show that a doubling of chromosomes occurs on the mother side when certain crosses are made between *Saccharum officinarum* (mother) and *Saccharum spontaneum*. The same phenomenon was observed in the cross *Vellai* × *S. spontaneum* by Dutt and Subba Rao [1933]. In the earlier years of sugarcane × sorghum hybridization at Coimbatore, attention was paid chiefly to the economic types obtained from this intergeneric hybridization, in other words, to those which more resembled the sugarcane. Chromosome counts of such hybrids indicated that in these also there was a doubling of chromosomes on the mother or sugarcane side [Singh, 1934].

Recently, types which showed more obviously sorghum characters—though not necessarily economic types—were also examined for chromosome counts. It was not possible to examine the pollen mother cells, as most of them were infertile and so an examination of root tips was made and the somatic numbers counted. The counts indicated that there was no doubling on the mother side.

Here then there would appear to be a case where, in the same F_1 generation, we have a doubling of chromosomes in certain of the progeny and no such doubling in others.

One hybrid of the latter class, which was pollen sterile, was back crossed with sorghum and the chromosome numbers of the resultant hybrids examined. The somatic numbers showed that it had two sets of sorghum chromosomes. Incidentally it may be mentioned that these " back crosses " showed a closer resemblance to sorghum in external morphology than any of the F_1 generation.

I am much indebted to Dr. Miss E. K. Janaki Ammal, Geneticist, for kindly going through the slides.

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Bremer, G. (1923). *Genetica* **5**, 97-148, 273-326.

Dutt, N. L. and Subba Rao, K. S. (1933). *Ind. J. Agri Sci.* **3**, 37.

Singh, T. S. N. (1934). *Ind. J. Agric. Sci.* **4**, 290.

CHROMOSOME NUMBERS IN SUGARCANE×*SORGHUM* HYBRIDS.

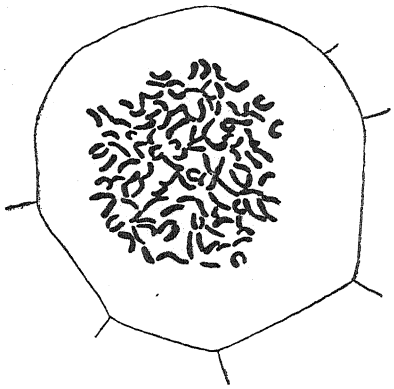


Fig. 1
Co. 351 (116 somatic)

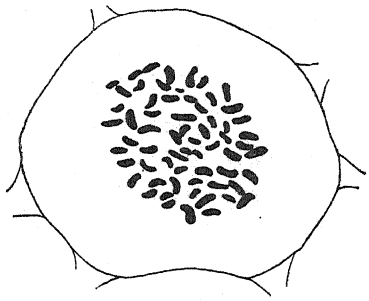


Fig. 2
S.C. 50 (64 somatic)

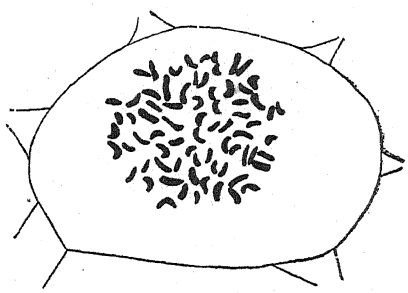


Fig. 3
G. 496 (73 somatic)

(Camera Lucida Drawings.)

Fig. 1. (Co. 351)=P. O. J. 2725 (106-108 somatic)×*Sorghum Durra* Stapf (20 somatic) showing $\frac{(53\text{-}54)+(53\text{-}54)+10}{2}\times 2$ or 116-118 somatic.

Fig. 2. (S. C. 50)=P. O. J. 2725 (106-108 somatic)×*Sorghum Durra* Stapf (20 somatic) showing $\frac{(53\text{-}54)+10}{2}\times 2$ or 63-64 somatic.

Fig. 3. (G. 496)=[P. O. J. 2725 (106-108 somatic)×*Sorghum Durra* Stapf (20 somatic)]×[*Sorghum Durra* Stapf (20 somatic)] showing $\frac{(53\text{-}54)+10+10}{2}\times 2$ or 73-74 somatic.

ERRATA

Indian Journal of Agricultural Science

(Vol. IV, Part III, June 1934.)

Plate XXX, *for* 'Fig. 4' *read* 'Fig. 2' and legends of Figs. 1 and 2, *for* '*Salvinia cucullata*' *read* '*Pistia stratiotes*'.

(Vol. IV, Part IV, August 1934.)

Page 715, line 12 from bottom, *for* 'larva' *read* 'larvae'.

Page 715, line 10 from bottom, *for* 'he' *read* 'she'.

Page 715, lines 9 and 7 from bottom, *for* 'his' *read* 'her'.

Page 716, line 14 *for* 'Boullion's' *read* 'Bouin's'.

Page 722, line 6, *for* 'extends' *read* 'extend'.

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